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UNIVERSITY OF ALBERTA

OZONE AND OZONE–HYDROGEN PEROXIDE INACTIVATION OF *GIARDIA*

BY

CHARLES W. LABATIUK ©

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND
RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

IN

ENVIRONMENTAL ENGINEERING

DEPARTMENT OF CIVIL ENGINEERING

EDMONTON, ALBERTA

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ABSTRACT

The Surface Water Treatment Rule requires a minimum of 99.9 percent inactivation or removal of *Giardia lamblia*. This study found that a minimum ozone residual of 0.5 mg/L and a contact time of 5 min will achieve this objective. Cohorts of five C3H/HeN mice were used to establish *Giardia muris* cyst infectivity. The mouse model was sensitive to 99.99 percent cyst inactivation and beyond.

Giardia muris cyst viability following ozonation was compared using fluorescein diacetate-ethidium bromide staining, the mouse model, and *in vitro* excystation. There was a significant difference between fluorogenic staining and infectivity ($P \leq 0.05$), with fluorogenic staining overestimating viability compared to infectivity. No significant differences were detected between infectivity and excystation, or between fluorogenic staining and excystation ($P \leq 0.05$) for inactivations up to 99.9 percent.

Ozone alone was a better disinfectant than ozone–hydrogen peroxide. A 0.05 M phosphate-0.01 M bicarbonate buffer, which has negligible ozone decomposition, produced significantly greater ($P \leq 0.05$) inactivation of *G. muris* cysts than did a 0.05 M phosphate buffer with peroxide system where ozone was completely decomposed in less than 60 s.

Factors affecting the inactivation of *G. muris* cysts using ozone were examined using 48 independent, experimentally designed trials in a bench-scale, batch reactor. Temperature, pH, applied ozone dose and the ozone dose x pH and temperature x pH interactions were found to be statistically significant ($P \leq 0.05$). Contact time in demand-free buffer was significant up to 2 min ($P \leq 0.05$), but not beyond. When experiments were done using natural surface waters, contact times up to 5 min were required for inactivation. It was more difficult to achieve 99.9 percent inactivation in the natural waters at 22°C than at 5°C. Residual and utilized ozone both had important influences on *G. muris* cyst inactivation. A nonlinear model was developed using these parameters.

Ozone inactivation of *G. muris* cysts was not adequately predicted by Ct (disinfectant concentration x contact time).

There were no apparent scale-up effects between bench- and pilot-scale natural water *G. muris* cyst inactivation results.

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LIST OF NOMENCLATURE

ANOVA	analysis of variance
AOP	advanced oxidation process
ATCC	American Type Culture Collection
AWWARF	American Water Works Association Research Foundation
BOD	biochemical oxygen demand, mg/L
β	regression coefficient
C	concentration of disinfectant, mass/unit vol
°C	degrees Celsius
C_{av}	average ozone, $[C_r + 0.5 \times (C_u - C_d)]$, mass/unit vol
C_d	ozone demand, mass/unit vol
C_o	disinfectant concentration at time 0, mass/unit vol
C_r	ozone residual, mass/unit vol
C_u	utilized ozone, mass/unit vol
CFU	colony forming units
COD	chemical oxygen demand, mg/L
CSTR	continuously stirred-tank reactor
Ct	concentration-time product, mg·min/L
Ct_{99}	Ct product giving 99 percent inactivation, mg·min/L
d.f.	degrees of freedom
DNA	deoxyribonucleic acid
DOC	dissolved organic carbon, mg/L
EB	ethidium bromide
ECW	empty cyst walls
exp.	experiment
ε	error, residual, deviation in observed dependent variable from its estimate

F	variance ratio
FDA	fluorescein diacetate
g	gram
GVRD	Greater Vancouver Regional District
h	hour
HPC	heterotrophic plate count
HDT	hydraulic detention time
I	number of infectious cysts per mouse after ozonation
IC	intact cysts
ID_{50}	median infectious dose
IFA	immunofluorescent assay
I_o	number of cysts in the inoculum in a control trial
IOA	International Ozone Association
k	reaction rate constant
log	base 10 logarithm
$\log (N/N_o)$	inactivation
M	molar concentration, moles/L
m	the fraction of a factorial design
mg/L	milligrams per liter
min	minute
$M\Omega$	megaohms
MS	mean square
MS_d	mean square error
MWDSC	Metropolitan Water District of Southern California
N	number of microorganisms after inactivation
n	number of positive mice in an ozonated trial; coefficient of dilution; number of factors
N_o	number of microorganisms before inactivation
n_o	number of positive mice in a control trial

NSR	North Saskatchewan River
NTU	nephelometric turbidity units
ω	oxidation competition coefficient
Ω_M	oxidation competition value of solute M, mg/L
P	probability
PBS	phosphate-buffered saline
PET	partially excysted trophozoites
pH	$-\log[H^+]$
PI	propidium iodide
ppm	parts per million
QA/QC	quality assurance/quality control
r.p.m.	revolutions per minute
s	second
SBH	Staehelin, Bühler and Hoigné aqueous ozone decomposition model
SD	standard deviation
spec. grav.	specific gravity
SPF	specific-pathogen-free
SWTR	Surface Water Treatment Rule
t	contact time, s or min
t_{10}	time such that 90 percent of organisms and liquid remains in ozone contactor at least t_{10} min
TE	total effect
TFG	Tomiyasu, Fukutomi and Gordon aqueous ozone decomposition model
THMFP	triholomethane formation potential
TOC	total organic carbon, mg/L
θ_1, θ_2, θ_3 and θ_4	nonlinear model parameters
u.v.	ultraviolet
USEPA	United States Environmental Protection Agency

V	volt
vol	volume
W	watt
w	specific ozone utilization rate, time^{-1}
wt	weight
X_i	general independent variable
y	dependent variable
y^*	model-predicted dependent variable
Y	general random variable

Cl_2	chlorine
ClO_2	chlorine dioxide
$CO_3^{\bullet -}$	carbonate radical ion
$HCO_3^{\bullet}$	bicarbonate radical
H_2O_2	hydrogen peroxide
H_3O^+	hydronium ion
$HO_2^{\bullet}$	hydroperoxide radical
HO_2^-	hydroperoxide anion
$HO_3^{\bullet}$	hydroozonide radical
HOCl	hypoclorous acid
I_2	iodine
NH_2Cl	monochloramine
${}^{\bullet}O^+$	oxygen cation radical
${}^{\bullet}O_2^-$	superoxide radical ion
O_3	molecular ozone
${}^{\bullet}O_3^-$	ozonide radical ion
${}^{\bullet}OH$	hydroxyl free radical
OH^-	hydroxide ion

1. INTRODUCTION

1.1 BACKGROUND

The 1986 Safe Drinking Water Act Amendments required the United States Environmental Protection Agency (USEPA) to regulate 83 contaminants including *Giardia lamblia*, other microbiological contaminants such as enteric viruses, *Legionella* and heterotrophic plate count bacteria, turbidity, volatile organic chemicals, synthetic organic chemicals, inorganic chemicals, and radionuclides (Regli, 1987; Rice, 1991). The microbiological and turbidity contaminants are regulated under the Surface Water Treatment Rule (SWTR), which requires all water systems serving 25 people or more and treating surface water to provide primary disinfection and filtration under specified water quality criteria by June 1993 (USEPA, 1989). The SWTR objectives for the inactivation or removal of *G. lamblia* cysts and enteric viruses are a minimum of 99.9 percent (3-log) and 99.99 percent (4-log), respectively. The primary disinfection requirements are summarized in Table 1.1. The filtration requirement can be waived provided the surface water turbidity is less than 5 NTU over any 12 consecutive months and there are less than 100 total coliforms per 100 mL or less than 20 fecal coliforms per 100 mL in 90 percent of samples collected over 6 months (USEPA, 1989). In addition, to avoid filtration, systems must meet other requirements such as a watershed control program, absence of waterborne disease outbreaks, and the maintenance of a 0.2 mg/L disinfectant residual in water entering the distribution system. Ozone is the main disinfectant capable of achieving the SWTR requirements in a reasonable plant contact time when filtration is not used.

The U.S. disinfection regulations are given in terms of the concentration x time (Ct) product and temperature (USEPA, 1991). The specific requirements for the inactivation of *G. lamblia* cysts by ozone are given in Table 1.2. Wickramanayake *et al.* (1984a; 1984b; 1985) and Wickramanayake and Sproul (1988) studied *Giardia muris*, *G. lamblia*, and *Nægleria fowlerii* in ozone demand-free buffer at bench-scale. Ct

Table 1.1 Primary disinfection requirements (USEPA, 1989)

Treatment Process	Log Removal or Inactivation of		Logs Filtration Removal Credit		Logs Removal or Inactivation Required of Primary Disinfectant	
	Giardia lamblia cysts	Enteric Viruses	*Giardia lamblia* cysts	Enteric Viruses	*Giardia lamblia* cysts	Enteric Viruses
Conventional Treatment	3	4	2.5	1	0.5	3
Direct filtration	3	4	2	1	1	3
No filtration	3	4	0	0	3	4

Table 1.2 Ct values (mg·min/L) for inactivation of *Giardia lamblia* cysts by ozone, pH 6–9 (USEPA, 1991)

Inactivation	Temperature (°C)					
$\log \frac{N}{N_o}$	<1	5	10	15	20	25
0.5	0.48	0.32	0.23	0.16	0.12	0.08
1	0.97	0.63	0.48	0.32	0.24	0.16
1.5	1.5	0.95	0.72	0.48	0.36	0.24
2	1.9	1.3	0.95	0.63	0.48	0.32
2.5	2.4	1.6	1.2	0.79	0.60	0.40
3	2.9	1.9	1.43	0.95	0.72	0.48

data from that research were extrapolated from 99 percent inactivation to 99.9 percent inactivation using the Chick-Watson model (Chick, 1908; Watson, 1908; USEPA, 1991) and was the source of the ozone inactivation criteria published in the Federal Register (USEPA, 1989). Extrapolating this type of data beyond observed experimental conditions can lead to erroneous conclusions (Hoff, 1987). The United States National Research Council (1980) adopted the Ct concept for comparing biocidal efficiency in their review of the disinfection literature; however, they recommended that comparisons be made at the 99 percent inactivation level to minimize problems associated with initial lags and "tailing off". The usefulness of the Ct system for ozone inactivation of *Giardia* has not been verified, and more information on ozone inactivation of *Giardia*, particularly at 99.9 percent and higher inactivation levels is required.

Since the work of Wickramanayake, a rigorous study has been done by the Metropolitan Water District of Southern California (MWDSC) (Wolfe *et al.*, 1989a; Wolfe *et al.*, 1989b; Scott *et al.*, 1992; Metropolitan Water District of Southern California and James M. Montgomery Consulting Engineers, 1991; Ferguson *et al.*, 1990), who used *in vitro* excystation for viability determination in a pilot-scale investigation of the disinfection efficacy of ozone–hydrogen peroxide and ozone in two untreated surface waters. It was found that ozone–hydrogen peroxide was less efficient at higher hydrogen peroxide to ozone ratios and this was postulated to be due to a concomitant lower ozone residual. These results suggest that the more active agent in the ozone–hydrogen peroxide process is molecular ozone rather than the hydroxyl radical. However, the chemistry of ozone in water is complex, and additional data on the disinfection performance of ozone in actual surface waters as well as more data on the advanced oxidation ozone–hydrogen peroxide process is required.

Chlorine is an effective and widely used disinfectant; however, the potential health risks from chlorination by-products have led to concerns regarding its use (Fawell *et al.*, 1987). The potential health effects of chlorine and combined chlorine compounds have been extensively researched (United States National Research Council, 1980; United States Department of Health and Human Services, 1986; Bull and Kopfler, 1991). The USEPA is currently developing a Disinfection –Disinfection By-product (D–DBP) Rule. Once this rule is promulgated, stringent new limits for total trihalomethanes may lead to an increase in the use of ozone. However, indications are that the USEPA will follow a two-phased regulatory approach, which will delay any major shift towards ozone use until the year 2000 (Rice, 1991). Noot *et al.* (1989), in an assessment of the Ames mutagenicity assay, noted that the amount of mutagenic material produced by ozone can sometimes be as high as that for chlorine. Ozone can also substantially increase assimilable organic carbon concentrations which would stimulate tremendous regrowth (Paszko-Kolva *et al.*, 1992). Research on ozonation by-products and analytical methods is ongoing (Glaze, 1986; Glaze *et al.*, 1989).

1.2 SCOPE OF RESEARCH PROGRAM

This research was motivated by the SWTR and was limited to ozone applied prior to filtration. The broad goals of this investigation were:

1. to develop engineering design criteria for *Giardia* using ozone and ozone–hydrogen peroxide; and
2. to extend knowledge on high levels of ozone inactivation of *Giardia* by using infectivity and animal models.

The program that evolved to achieve the above goals included:

1. the review of relevant literature on *Giardia* and ozone;
2. development of procedures involved in the use of animal models for *Giardia* following ozonation;
3. the design and analysis of ozonation experiments of *Giardia* in laboratory and natural surface waters; and
4. evaluation of the existing Ct system and development of an alternative process design protocol.

2. LITERATURE REVIEW

2.1 *GIARDIA*: THE ORGANISM

2.1.1 Taxonomy and Morphology

Giardia is a multi-flagellated protozoan and its currently accepted taxonomic classification is given in Table 2.1. Approximately 40 species of *Giardia* have been described using either host specificity or morphology as criteria (Meyer and Radulescu, 1979). There is considerable confusion regarding the nomenclature of this protozoan. *Giardia* is used in the Western World, while the name *Lamblia* is more frequent in Eastern European countries. Various names used for *Giardia* from man are *Giardia duodenalis, Giardia intestinalis, Giardia lamblia,* and *Giardia enterica* (Lin, 1985; Erlandsen *et al.*, 1988). Filice (1952) concluded that the use of body dimensions and host specificity were unreliable and subdivided the genus into three distinct morphological groups based on the shape and position of the median body (microtubular organelles within the trophozoites):

1. *Giardia agilis* group: These organisms are parasites of tadpoles and frogs. Their median bodies are long and teardrop-shaped and are parallel to the longitudinal axis of the cell body.

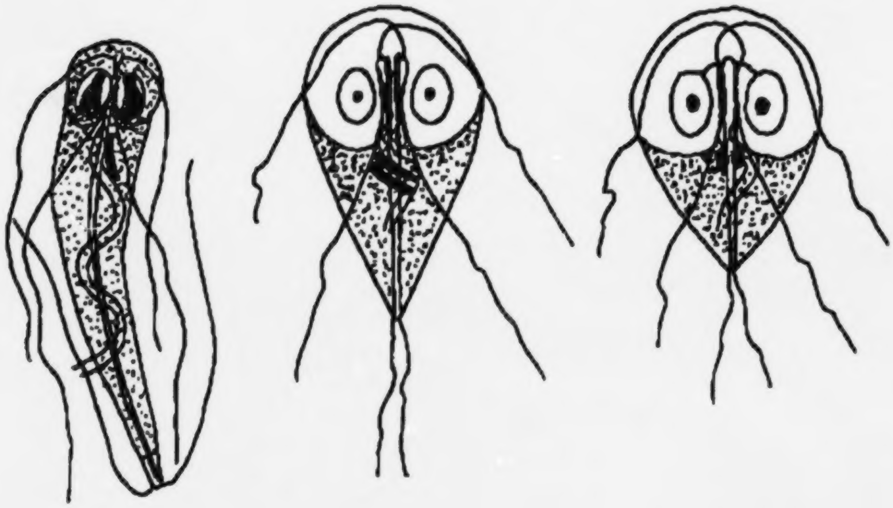
2. *Giardia duodenalis* group: These are parasites of a variety of warm-blooded vertebrates including humans and rodents. They have single or double claw-shaped median bodies that are situated transversely in the mid-portion of the cell body.

3. *Giardia muris* group: These are parasites of rodents and birds. They have two small, round median bodies that are located in the center of the cell body.

The relative differences of the trophozoites of these three species is indicated in Figure 2.1. Recent chemotaxonomic advances offer

Table 2.1 Taxonomic classification of *Giardia* (Levine, 1979; Meyer, 1990)

Taxonomic Group	Classification
Phylum	Protozoa
Subphylum	Sarcomastigophora
Superclass	Mastigophora
Class	Zoomastigophorasida
Order	Diplomonadorina
Family	Hexamitidae
Subfamily	Octomitinae
Genus	*Giardia*



G. agilis **G. duodenalis** **G. muris**

Figure 2.1 Morphological groups of *Giardia* spp. (after Filice, 1952)

potential in being able to differentiate between species in the various groups of *Giardia* (Meyer, 1990).

2.1.2 Biology

There are two stages in the life cycle of *Giardia* in warm-blooded vertebrates: the trophozoite and the cyst (Figure 2.2). The trophozoite commonly inhabits the duodenum while the cyst is found in the small and large intestine and the feces. The trophozoite is a bilaterally symmetrical, pear-shaped organism with two nuclei and four pairs of flagella. The dimensions of trophozoites vary between morphological groups as well as in different isolates within a group. *Giardia lamblia* is about 12 to 15 µm long and 6 to 8 µm wide. *Giardia muris* is approximately 9 to 12 µm long and 5 to 7 µm wide. The trophozoite of *Giardia agilis* is long and narrow, about 20 to 30 µm long and 4 to 5 µm wide. Trophozoites divide by longitudinal binary fission and disintegrate outside the host in the external environment.

Giardia has a direct life cycle and the cyst is the normal infective stage in the transmission of giardiasis to new hosts. *Giardia* cysts are ovoid in shape and have a length of 8 to 12 µm and a diameter of 7 to 10 µm depending on the species (Levine, 1979). The cyst wall varies from 0.3 to 0.5 µm in thickness and electron microscopy has shown it to be composed of thin fibrous elements interspersed with fine particles (Sheffield, 1979). The chemical nature of the cyst wall is not understood. One researcher reported that the cyst wall of *G. lamblia* and *G. muris* type organisms contain chitin, while another, using gas chromatography and mass spectrometry, demonstrated that *n*-acetylgalactosamine is the primary amino sugar of the cyst wall, but was unable to detect *n*-acetylglucosamine, the primary amino sugar of chitin (Feely *et al.*, 1990). Cysts have a maturation period. Young cysts have two nuclei, while mature cysts have four. After emergence from the cyst, the organism undergoes cytokinesis within 5 to 30 minutes after the initiation of excystation. Thus, each mature cyst yields two trophozoites.

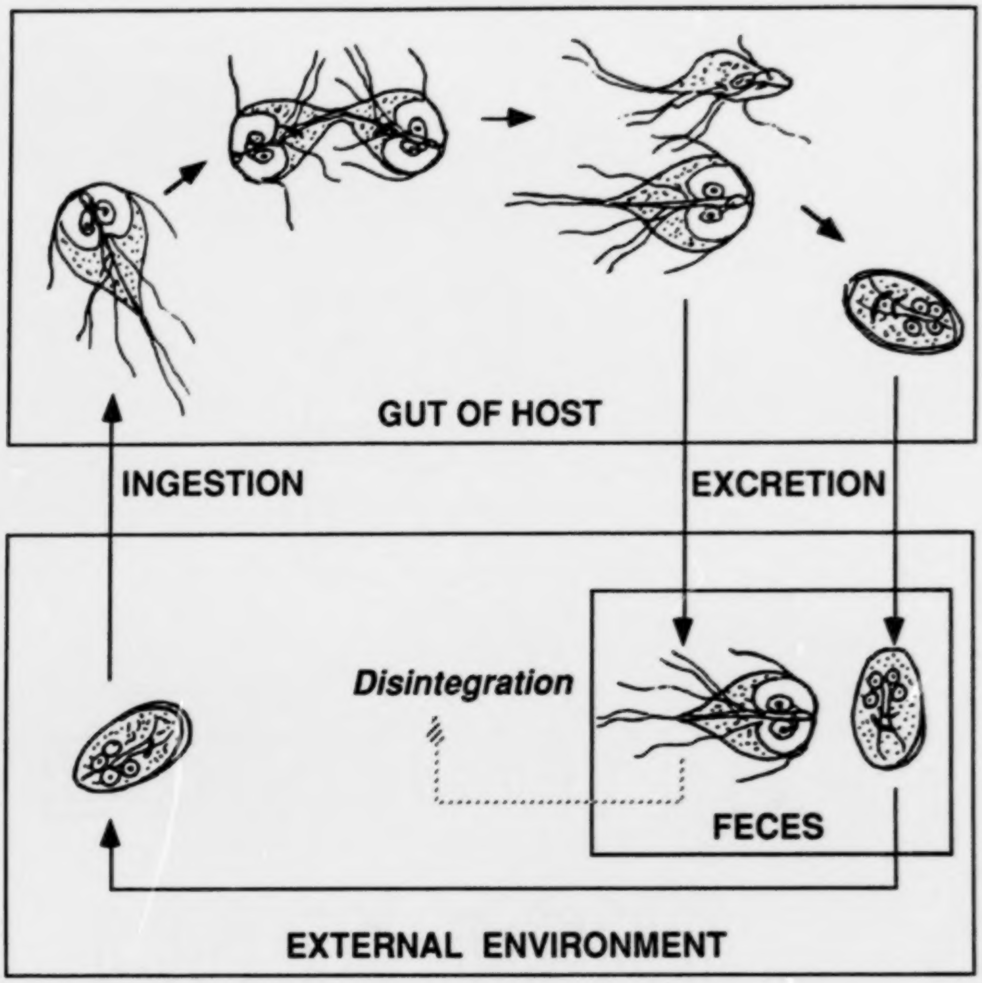

GUT OF HOST
INGESTION
EXCRETION
Disintegration
FECES
EXTERNAL ENVIRONMENT

Figure 2.2 Life cycle of *Giardia* spp. (adapted from Meyer and Jarroll, 1980)

The ability of *Giardia* trophozoites to attach to the wall of the small intestine of host organisms is one of their most significant biological functions. At least five hypotheses on the mechanism of attachment have been proposed (Belosevic, 1985):

1. negative pressure under the ventral disk produced by movement of the ventral flagella;
2. contractile activity of the ventrolateral flange resulting in attachment ability;
3. a combination of negative pressure and contraction of the ventrolateral flange;
4. microtubular coiling-uncoiling; and
5. attachment mediated by contractile proteins.

Since the fourth hypothesis was proposed, it has been demonstrated that a necessary prerequisite was lacking, and thus the coiling-uncoiling model has been refuted (Belosevic, 1985). *G. lamblia* trophozoites can be grown axenically (Meyer and Radulescu, 1979; 1984; Meyer, 1979), and Gillin (1984) demonstrated the role of the thiol-reducing agent cysteine in attachment of trophozoites to the walls of culture vessels and also in protecting trophozoites from the lethal effects of oxygen. Kaplan and Altmanshofer (1985) observed that parasite adherence to the small intestine appeared necessary for the perpetuation of infection, and developed a *G. muris* trophozoite adherence model to examine the effects of serum and milk antibodies on parasite attachment.

Further information on the biology of *Giardia* can be found elsewhere (Levine, 1979; Meyer and Radulescu, 1979; Feely *et al.*, 1990).

2.1.3 Detection

Detection of *Giardia* cysts in water involves four distinct steps:

1. sample collection from an appropriate volume of water;
2. sample extraction, usually by filter elution;

3. sample concentration and clean up; and
4. parasite detection, usually by microscopic observation.

Considerable advances have been made in these steps since 1975, however there is no cultural method for the detection of *Giardia* cysts in water (Jakubowski, 1984). The reference method is regarded as "experimental" and involves filtering 380 L of water through a yarn-wound microporous filter, concentration by zinc-sulphate flotation, and microscopic examination (APHA *et al.*, 1989). Membrane filters were used by Wallis and Buchanan-Mappin (1985) and Ongerth (1989) who reported cyst recoveries of 53 percent and 22 percent, respectively. Payment *et al.* (1989) reported 71 percent cyst recovery after elution from a wound filter, and a final recovery of 52 percent after low-speed centrifugation and sucrose density gradient flotation.

Microscopic identification involves observing organisms of the right size and shape and having at least two internal features such as nuclei, median bodies, and axonemes visible (APHA *et al.*, 1989). Because microscopic identification requires a skilled observer and internal morphological characteristics can be destroyed by disinfectants, fluorescent antibody staining techniques may be more suitable (Sauch and Berman, 1991; Engelkirk and Pickering, 1990). Sauch (1985) reported on the use of an indirect immunofluorescense/phase-contrast microscopy method for the detection of *Giardia* cysts in finished and heavily contaminated raw surface water samples. Stetzenbach *et al.* (1988) reported on a monoclonal antibody based immunofluorescent assay (IFA) and Rose *et al.* (1989) evaluated the use of membrane filtration and monoclonal and polyclonal antibodies for *Giardia* cysts detection in environmental samples. Use of IFA techniques can result in false-positives. Algae, diatoms and pollen may cross-react with the reagents used and can be of similar size and shape as the target organism.

Ideally, after sample processing the concentrate would contain all the cysts present in the original sample and no extraneous debris (Jakubowski, 1984). Interfering debris can make microscopic examination tedious. Recent developments such as magnetic antibody methods (Bifulco and Schaefer, 1990) and gene-specific probes (Abbaszadegan *et al.*, 1991) mitigate the effects of debris. Gene probes are able to detect the presence of low numbers of *Giardia* cysts, however they do not provide quantitative data and at present are not commercially available. Further information on the emerging technology of gene probes can be found elsewhere (Richardson *et al.*, 1991).

2.1.4 Occurrence in Natural Waters

Giardia spp. cyst concentrations in three Washington state watersheds were investigated (Ongerth, 1989). *Giardia* spp. cysts were found in 43 percent of 222 samples, with cyst concentrations ranging from 0.1 to 5.2 cysts/L. Another study of a western United States watershed determined that there was no correlation between total or fecal coliforms or turbidity and *Giardia* (Rose *et al.*, 1988). However *Giardia* and *Cryptosporidium* were found to be significantly correlated with each other. A study of the occurrence of *Giardia* spp. cysts in the United Kingdom analyzed 263 environmental samples and found that 22 percent of treated water samples, 46 percent of raw potable water samples, 85 percent of recreational waters and all sewage effluents contained cysts. Mean cyst concentrations were 0.08, 0.14, 1.74 and 359 cysts/L, respectively (Gilmour *et al.*, 1991).

A waterborne *Giardia* epidemic in Pennsylvania prompted an evaluation of monitoring wastewater for *Giardia* as a preventative measure and detection tool in watersheds contaminated by sewage (Sykora *et al.*, 1986). While the highest cyst concentration in raw Youghiogheny River water was 1.2 cysts/L, the cyst concentration in raw wastewater ranged from 11.1 to 1.3×10^3 cysts/L. It was estimated that the USEPA procedure resulted in 3 to 14 percent recovery of *Giardia* cysts in raw wastewater samples by performing direct counts in addition to the USEPA procedure (Sykora *et al.*, 1986). A seasonal distribution of *Giardia* cysts in wastewater has been reported, with the highest concentrations occurring in late summer, fall and early winter in the U.S., or in late winter in France (Sykora *et al.*, 1991; Gassmann and Schwartzbrod, 1991). No correlation was found between geographical location and *Giardia* cyst concentration in a one year survey involving 11 wastewater treatment plants located across the U.S. (Jakubowski *et al.*, 1991).

An extensive survey of the source waters of 66 surface water treatment plants in 14 eastern states and one Canadian province found *Giardia* spp. to be widely dispersed in the aquatic environment (LeChevallier *et al.*, 1991; 1992). The IFA detection procedure used had an average recovery of 48 percent of *Giardia* cysts in raw water samples with turbidities up to 150 NTU. *Giardia* cysts were found in 81 percent of the raw water samples, with cyst densities up to 10 times higher for source waters receiving industrial or sewage effluents than for protected watersheds. *Giardia* cyst densities and total and fecal coliform levels in the waters were significantly correlated ($P < 0.01$). This is contrary to the results of others (Akin and Jakubowski, 1986; Rose *et al.*, 1988); however, the previous studies primarily examined pristine source waters.

2.1.5 Viability Measurement

The detection of *Giardia* spp. cysts in environmental samples does not imply that the organisms are viable and will infect animals; rather, one of the currently available methods must be used for viability determination (deRegnier *et al.*, 1989). These methods include vital dye exclusion (Bingham *et al.*, 1979), fluorogenic dye staining (Hale *et al.*, 1985; Jackson *et al.*, 1985; Jones and Senft, 1985; Schupp *et al.*, 1988), *in vitro* excystation (Bingham and Meyer, 1979; Schaefer *et al.*, 1984; Schaefer, 1990), animal infectivity (Roberts-Thomson *et al.*, 1976a; Belosevic and Faubert, 1983a; Belosevic *et al.*, 1983), and cyst morphology by light microscopy (Schupp and Erlandsen, 1987a). No single method has been accepted as a reference method, however *in vitro* excystation is the most frequently used and has been recommended by the USEPA for use in chemical disinfection studies (USEPA, 1991).

The basic difficulty with all methods is procedural. A viable cell is capable of DNA replication, metabolizing, and selectively excluding molecules. Vital dyes, particularly eosin staining, were one of the first measures of *Giardia* cyst viability. However, the development of *in vitro* excystation showed eosin to be unreliable because it overestimated the resistance of *G. lamblia* cysts to chlorine (Bingham *et al.*, 1979). Of ultimate interest is the ability of the organism to cause an infection. Animal models are the only method that provides a direct measurement of the ability of cysts to cause an infection. Other methods give no information on infectivity. Disadvantages of animal models include their expense and time consuming nature. The Mongolian gerbil-*G. lamblia* model has additional challenges because cyst release is intermittent and the infectious dose uncertain. A representation of the relationship between the various viability methods is shown in Figure 2.3. The smallest area for infectivity indicates that it is the most sensitive method. All infective cysts would have shown viability by the other methods; however, not all cysts shown to be viable by fluorogenic dye staining will have shown viability by excystation (deRegnier *et al.*, 1989).

Using fluorogenic stains as measures of *Giardia* cyst viability is a recent development of much interest because the procedures are inexpensive, simple to use and provide immediate results (Hale *et al.*, 1985; Schupp and Erlandsen, 1987b; Ongerth *et al.*, 1989; deRegnier *et al.*, 1989; Sauch and Berman, 1991). Fluorogenic stains such as fluorescein diacetate (FDA) and ethidium bromide (EB) or propidium iodide (PI) measure viability by the metabolic conversion or selective exclusion of the stain. Live cysts metabolize FDA to fluorescein and the cyst perimeter fluoresces green when viewed under a fluorescent microscope. Dead cysts are unable to metabolize FDA; however, only dead or dying cysts are able to absorb PI or EB, which binds with nucleic acids, causing orange to bright red fluorescense. One problem is that some fluorogenic stains are affected by the particular disinfectant used because killing a cyst may affect binding sites or cyst walls. This was the case with PI and chlorine (Sauch *et al.*, 1991). Another potential problem is that fluorogenic stains may work with one pathogen but not another, i.e. FDA works for *Giardia* but not *Cryptosporidium*. As well, there may be gradations in staining intensity and double staining procedures have shown that there is a population of cysts that does not stain at all (Schaefer, 1990). This unstained population was less than 10 percent with *G. muris* cysts but was 100 percent in one of four *G. lamblia* isolates using FDA-PI in studies that did not involve disinfectants (Schupp and

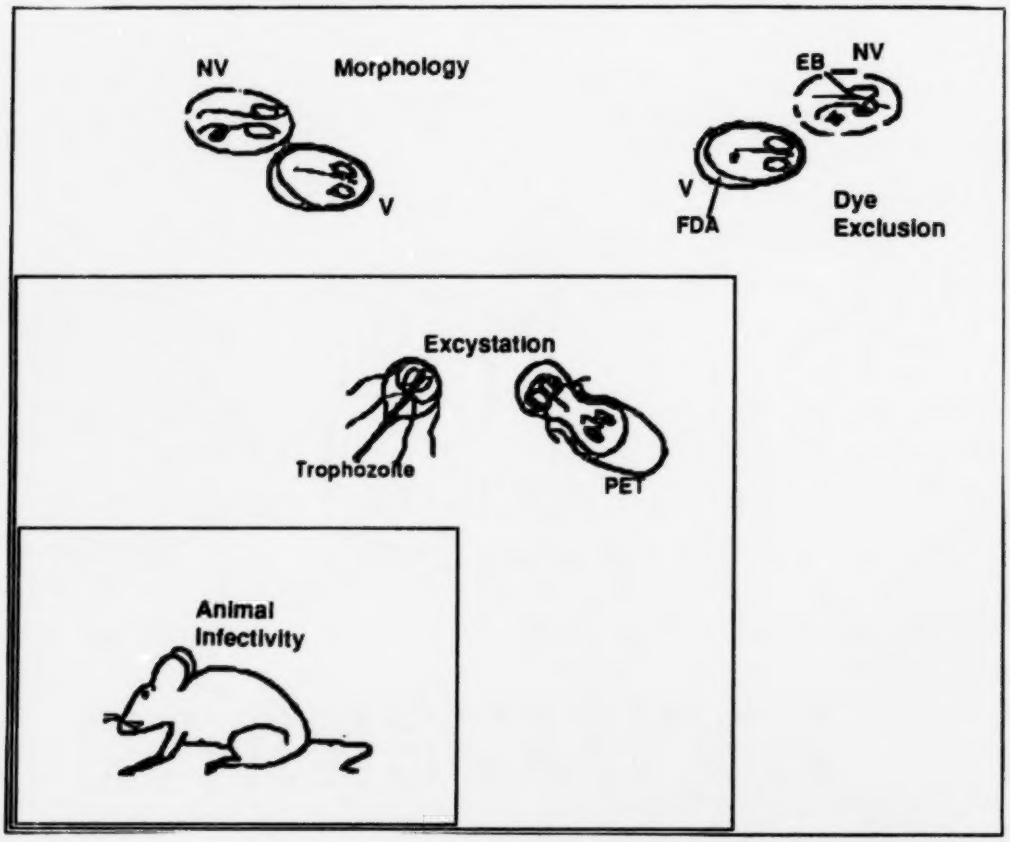

NV
Morphology
V
EB
NV
V
FDA
Dye Exclusion
Excystation
Trophozoite
PET
Animal Infectivity

Figure 2.3 Relationship between *Giardia* cyst viability methods. FDA, fluorescein diacetate; EB, ethidium bromide; PET, partially excysted trophozoite; V, viable; NV, nonviable. Smaller box indicates more sensitive viability method. (adapted from deRegnier *et al.*, 1989)

Erlandsen, 1987b; Smith and Smith, 1989). A disinfection study using 30 min exposure to 1 mg/L chlorine found that 21 percent of *G. lamblia* cysts were unstained with FDA-EB (Hale *et al.*, 1985).

Excystation is a chemical procedure involving three steps: induction at low pH, washing and sedimentation, followed by an incubation step in which an excystment medium is used. The optimal induction pH for both *G. lamblia* and *G. muris* is about 2, mimicking the mammalian stomach, which ranges between pH 1.5 and 2.5 (Meyer and Schaefer, 1984). Excystation requires active participation of the parasite, which is assumed to be viable to excyst. While *G. muris* excystation levels are consistently greater than 90 percent, *G. lamblia* excystation efficiencies are much less than 90 percent and highly variable (Schaefer, 1990). *G. lamblia* excystation procedures have been reported to be nonreproducible (Wickramanayake, 1984), in the order of 50 to 60 percent (Jarroll, 1988), and at a mean level of 87 percent for symptomatic donors and 70 percent for asymptomatic donors (Meyer and Schaefer, 1984). Variations in cyst preparation or the specific excystation procedure and strain of *G. lamblia* used may account for some of these differences. Excystation procedures for *Giardia* spp. presently require between 1×10^5 and 5×10^5 cysts (Schaefer *et al.*, 1984). It is necessary to examine microscopically 1,000 cysts to measure 99.9 percent inactivation. However only 0.2 to 1 percent of the initial population is being assessed. The implications regarding the accuracy and precision of inactivation quantitation are unknown (Schaefer, 1990). Sauch (1988) reported on an excystation procedure using enzyme-free media. The excystment medium of trypsin-Tyrode's solution was replaced with Hanks' balanced salt solution supplemented with cysteine, sodium bicarbonate and proteose peptone or phytone peptone for *G. muris* and *G. lamblia*, respectively. The results were comparable with methods utilizing trypsin. Schupp and Erlandsen (1987b) noted that excystation is subjective because aborted attempts at trophozoite emergence might be included. In determining excystation for *G. lamblia*, mobile trophozoites are counted, and care must be taken to determine whether the excysted trophozoites are alive or dead (Schaefer, 1990).

Infectivity can be determined by using animal models (Faubert and Belosevic, 1990) including the mouse-*G. muris* model (Roberts-Thomson *et. al.*, 1976a; 1976b; Belosevic and Faubert, 1983a; 1983b; Belosevic *et. al.*, 1984; Faubert *et. al.*, 1985) and the Mongolian gerbil (*Meriones unguiculatus*)-*G. lamblia* model (Belosevic *et. al.*, 1983; Faubert *et. al.*, 1983; Lewis *et al.*, 1987). An animal model involves both parasite factors and host factors. Belosevic (1985) summarized the criteria for an ideal animal model for human giardiasis:

1. the host must be highly susceptible to infection;
2. trophozoites must be able to multiply in the small intestine;
3. cyst formation and release must occur;
4. the infection must be transferable through oral inoculation;
5. it must be possible to quantitate the intensity of infection;
6. species which cause giardiasis in humans should be used;
7. the host should be inexpensive, easily maintained and widely available; and
8. the course of infection and concomitant pathology in the host animal should mimic that observed in human giardiasis.

The mouse-*G.muris* model was originally described by Iwanczuk (1968) and Roberts-Thomson *et. al.* (1976a) and the correlation between oral dosage and course of infection was further defined by Belosevic and Faubert (1983a). Parameters of the mouse-*G.muris* model include the latent period and trophozoite burden in the small intestine. These parameters permit an accurate assessment of infective cyst dose.

The Mongolian gerbil-*G. lamblia* model was proposed by Belosevic *et al.* (1983) and its characteristics include: high susceptibility to infection with *G. lamblia* cysts, a pattern of cyst release and duration of infection similar to that for the human infection, and the nontransient nature of the infection as demonstrated by an increase in trophozoite colonization and concomitant cyst formation at higher levels than the original inoculum. The latent period varied from 4 to 8 days depending on the inoculum size, and the duration of infection was 13 to 86 days; however, most cysts were observed during the second and third weeks. Cyst release was intermittent and it has been concluded that this is a feature of the parasite, rather than a host characteristic (Belosevic *et al.*, 1983; Faubert *et al.*, 1983). Mongolian gerbils acquire complete resistance when challenged by the same species of *Giardia*, but only partial resistance when challenged with a different species of *Giardia*, and can therefore be used for the identification of the etiological agent as well as determining infectivity (Faubert *et al.*, 1983).

The median infectious dose (ID_{50}) has been reported to be approximately 282 to 316 cysts for *G. lamblia* in the Mongolian gerbil model (Schaefer *et al.*, 1991). Rendtorff (1954), in a human feeding experiment on adult male volunteers in a correctional institution in Seagoville, Texas, concluded that as few as 10 cysts was an infectious dose for *G. lamblia*. However, while 2 of 2 volunteers fed 10 cysts became positive, only 6 of 20 volunteers fed 25 cysts were positive (30 percent), and 2 of 2 volunteers fed 100 cysts were positive (Rendtorff, 1954). Furthermore, the criterion used for an infected subject was cysts observed in the feces because none of the subjects developed clinical giardiasis (Rendtorff, 1954). Visvesvara *et al.* (1988) reported that only certain isolates of human-derived *G. lamblia* infect Mongolian gerbils and that the ID_{50} was at least 100 cysts. Uncertainty regarding the number of cysts in an infectious dose and the intermittent pattern of cyst release can make numerical determination of inactivation imprecise using the Mongolian gerbil model. Rather the model should be used to determine if infection has occurred. This typically has a yes or no answer. The use of median infected dose would give the most statistically valid quantitation of inactivation; however, this method would require the serial dilution of treated cysts and numerous animals. The result would be very similar to most probable number methods (APHA *et al.*, 1989; Haas and Heller, 1990).

Determination of viability by cyst morphology requires a skilled observer. Viable FDA-positive cysts have well defined cyst walls, a peritrophic space, and flagella at one pole. Nonviable EB-positive cysts do not have a peritrophic space however cytoplasmic organelles (axonemes, median bodies, and nuclei) are seen (Schupp and Erlandsen, 1987a; Schaefer, 1990).

A number of researchers have compared two or more of fluorogenic stains, *in vitro* excystation and animal infectivity in studies that did not involve disinfectants. DeRegnier *et al.* (1989) used PI exclusion and infectivity in non-Swiss CF-1 mice to measure the viability of *G. muris* cysts suspended in lake, river, and tap water. They concluded that *G. muris* cyst viability as determined by mouse infectivity could not be directly compared with PI exclusion because as few as 5 to 10 cysts may have been an infective dose, while the inocula used in their study may have contained from 10^2 to 10^3 cysts. Schupp and Erlandsen (1987b) found that FDA-positive cysts were viable, as determined by infectivity, and that PI-positive cysts were nonviable and incapable of producing infection. By using 10^3 FDA-positive cysts as the inoculum, cysts were detected on day 5; with an inoculum size of 5×10^4, cysts were detected on day 3. Smith and Smith (1989) reported that FDA staining of *G. lamblia* consistently overestimated cyst viability and PI staining underestimated viability compared with *in vitro* excystation. Schupp *et al.* (1988) used FDA-PI, *in vitro* excystation and mouse and gerbil giardiasis models to determine the viability of *in vitro*-produced *Giardia* cysts. Viability was 48.7 percent by FDA-EB, less than 1 percent by excystation; infectivity was 37 percent (n=8) in the gerbil model, 0 percent (n=6) in neonatal mice and 30 percent (n=10) in suckling mice.

A comparison of animal infectivity and excystation as measures of *G. muris* cyst inactivation by chlorine was made by Hoff *et al.* (1985). They determined that 1 to 15 *G. muris* cysts constituted an infectious dose for outbred, female, Swiss CF-1 mice, with 5 cysts being the ID_{50} (mean of 10 trials). Neither animal infectivity nor excystation consistently showed a higher degree of inactivation. However, only inactivations up to 99.7 percent were examined. Fluorogenic staining techniques have been used to assess *Giardia* cyst viability following chlorination (Hale *et al.*, 1985), and to evaluate the efficacy of iodine- and

Table 2.2 Previous viability studies using fluorogenic stains or animal infectivity.

Reference	Parasite	Stain	Host	Disin-fectant
Jackson *et al.* (1985)	*Leishmania*	FDA-EB	-	-
Hale *et al.* (1985)	*G. lamblia*	FDA-EB	-	Cl_2
Schupp & Erlandsen (1987b)	*G. muris*	FDA-PI	-	-
deRegnier *et al.* (1989)	*G. muris*	PI	CF-1 non-Swiss mice	-
Smith & Smith (1989)	*G. intestinalis*	FDA-PI	-	-
Ongerth *et al.* (1989)	*G. lamblia*	FDA-EB	-	I_2, Cl_2
Sauch *et al.* (1991)	*G. lamblia*	PI	-	-
	G. muris	PI	-	Cl_2, NH_2Cl, BTA*
Hoff *et al.* (1985)	*G. muris*	-	female CF-1 Swiss mice	Cl_2
Hibler *et al.* (1987)	*G. duodenalis*	-	Mongolian gerbil	Cl_2
Wallis *et al.* (1990)	*G. duodenalis* *G. muris*	-	Swiss Webster mice	ClO_2 O_3

*BTA, benzyldimethyltetradecyl ammonium;
FDA, fluorescein diacetate;
EB, ethidium bromide;
PI, propidium iodide.

chlorine-based backcountry water treatment devices (Ongerth *et al.*, 1989). Hale *et al.* (1985) reported that *G. lamblia* cysts ceased to stain positively with FDA only at toxic concentrations of chlorine (256 mg/L) with a contact time of 30 min. Ongerth *et al.* (1989) reported that fluorogenic staining indicated a slightly higher proportion of viable *G. muris* cysts than did *in vitro* excystation following heat inactivation.

The majority of disinfection studies have used *in vitro* excystation for viability determination (Jarroll, 1988). Results have generally been presented as qualitative observations rather than rigorous statistical comparisons. Table 2.2 outlines previous studies in which fluorogenic dyes and animal infectivity were used. No studies investigating the use of fluorogenic staining following ozonation were found.

2.2 GIARDIASIS: THE DISEASE

The intestinal disease caused by the protozoan parasite *G. lamblia* is termed giardiasis. It was only in the 1970's that *Giardia lamblia* became generally recognized as a pathogenic protozoan parasite (Healy, 1979; Tombes *et al.*, 1979). Consequently much of the earlier ozone disinfection literature, such as Newton and Jones (1949), considers only *Entamoeba histolytica*. *G. lamblia*, and more recently *Cryptosporidium parvum*, are only "new" in the sense of recognition of their individual or collective pathogenicity. Giardiasis has also been termed "traveler's disease", and as the public and press have become more aware of it, "backpacker's diarrhea" and "beaver fever" (Feely *et al.*, 1990). Since 1970, when travel to the Soviet Union increased, Leningrad has been implicated as an endemic source of *Giardia* to travelers, and the probable mode of transmission is hotel tap water (Lin, 1985).

In the U.S., during the period 1971–85 *G. lamblia* was the most commonly determined cause of waterborne disease outbreaks in terms of both the number of outbreaks and the number of persons affected (Craun, 1988; 1990; Craun and McGoldrick, 1990; Levine *et al.* 1990). However, an etiologic agent was determined in only 50 percent of the waterborne outbreaks during this period. The remaining outbreaks were categorized as gastrointestinal illness due to unidentified agents (Craun, 1988).

2.2.1 Prevalence

The percentage of asymptomatic carriers of *G. lamblia* is estimated to be between 1 and 7 percent in industrialized countries (Dupont and Sullivan, 1986). Prevalence is especially high in places where hygiene practices may be poor including day-care centers and institutions for the mentally retarded (Healy, 1979; Lin, 1985). Giardiasis is endemic in the Canadian Arctic and a prevalence as high as 72 percent has been reported for Greenland populations (Foliguet *et al.*, 1987). The prevalence of giardiasis among U.S. children is 4 to 22 percent, slightly higher than the 2 to 15 percent prevalence among U.S. adults (Healy, 1979).

The carriage rate of *G. lamblia* is estimated to range from 5 to 50 percent in developing countries (Dupont and Sullivan, 1986). Giardiasis is most prevalent in poor communities with inadequate water supply and sanitation (Feachem *et al.*, 1983). The prevalence of *G. lamblia* was 15 to 30 percent in a study of four selected highland Costa Rican villages (Mata, 1986) and 36 percent in a community of 6 to 20 year old males living in an orphanage near Mogadishu, Somalia (Ilardi *et al.*, 1986).

In Canada there were approximately 9,000 cases of giardiasis from all sources yearly during the period 1987–90 (Health and Welfare Canada, Laboratory Centre for Disease Control, Ottawa, Ont., 1992, Personal communication). Yukon had the highest number of cases per hundred thousand population (Figure 2.4), with the number of cases in 1990 due in part to an outbreak in Carcross. In the U.S., the total reported cases of waterborne giardiasis was 24,124 during 1971–85. These cases were from 92 outbreaks, or 37 percent of the waterborne outbreaks with an established etiology during this period (Craun, 1990). Most of these outbreaks (58 percent) occurred during 1980–85; however, this is likely due to greater voluntary reporting and improved detection capabilities.

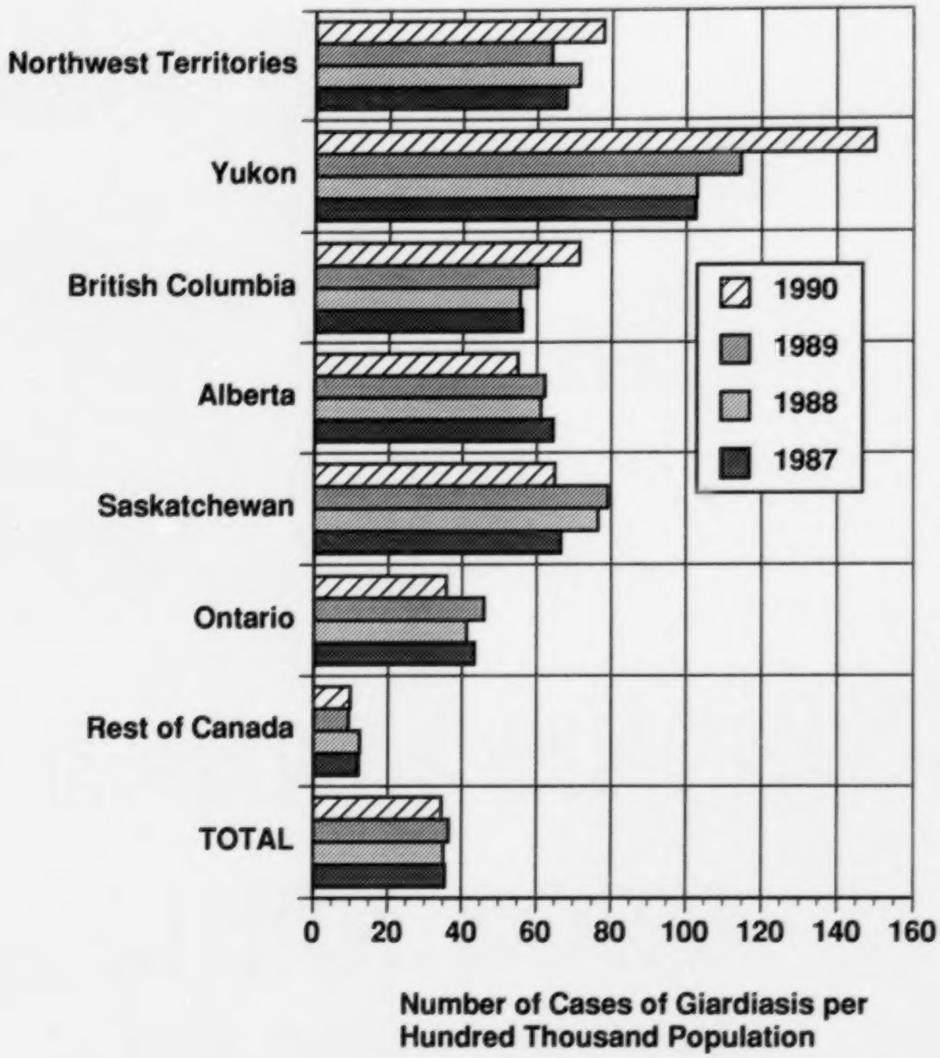


Figure 2.4 Total reported cases of giardiasis per hundred thousand population in Canada, 1987–90 (Health and Welfare Canada, Laboratory Centre for Disease Control, Ottawa, Ontario, 1992, Personal communication)

Domestic and wild animals can act as reservoirs of *Giardia*, and cross-species transmission of *Giardia* spp. between humans and a variety of animal species has been reported (Foliguet *et al.*, 1987; Craun, 1990). During 1982 and 1983 it was determined that *Giardia* spp. was the most common parasite of dogs in Alberta, Canada, with an overall incidence of 8 percent (Lewis, 1984). The prevalence of *Giardia* spp. in dogs from an Edmonton animal shelter was five times greater in February 1983 than during the previous summer when there was no human outbreak. Although beavers have been implicated as the source of waterborne outbreaks (Kirner *et al.*, 1978; Dykes *et al.*, 1980), the prevalence of *Giardia* spp. in beavers and muskrats can be high and show little variation among geographic regions, limiting the usefulness of animal stool surveys in assessing the risk of *Giardia* in water supplies (Craun, 1990). The prevalence of *Giardia* spp. in four northeastern states and Minnesota was 95.9 percent and 13.7 percent in live-trapped muskrat and beaver populations, respectively, determined by detection of intestinal trophozoites at necropsy; and 36.6 percent in muskrats and 9.2 percent in beavers, determined by detection of cysts in fecal samples from kill-trapped animals (Erlandsen *et al.*, 1990).

2.2.2 Epidemiology

There are two main routes of transmission: the fecal-oral route and the waterborne route. There have also been several documented cases of transmission through water contact in swimming pools (Porter *et al.*, 1988; Harter *et al.*, 1984). The incidence of giardiasis is high in a number of situations involving person-to-person contact including day-care centers, institutions for the mentally retarded, and among male homosexuals (Lin, 1985). Extensive literature is available on waterborne giardiasis outbreaks (Craun, 1990; Akin and Jakubowski, 1986). Some of the best-documented North American waterborne outbreaks of giardiasis include those of Rome, New York in 1974-75 (Shaw *et al.*, 1977); Camas, Washington in 1976 (Kirner *et al.*, 1978; Dykes *et al.*, 1980); Berlin, New Hampshire in 1977 (Lippy, 1978); Banff, Alberta in 1982 (Wilson *et al.*, 1982); and Edmonton, Alberta in 1982-83 (Collier and MacDonald, 1983;

Lippy, 1984; McClure and Mackenzie, 1988). Characteristics of these outbreaks are summarized in Table 2.3.

The Rome, New York giardiasis outbreak was the largest ever reported in the U.S. and also was the first time that *G. lamblia* cysts were actually recovered from treated water (Shaw *et al.*, 1977). The Camas, Washington outbreak is noteworthy as being the first involving a filtered water supply and the first time that an animal reservoir was implicated as a source of water contamination (Kirner *et al.*, 1978; Dykes *et al.*, 1980). *G. lamblia* cysts were detected in the raw surface water and in samples from the distribution system. In Alberta there have been two important outbreaks of giardiasis (Table 2.3). At the time of the Banff outbreak, it's water supply came from a protected watershed; however beaver were found to have colonized the watershed. A boil-water order was issued until Banff could convert to a groundwater source. The majority of cases in the Edmonton outbreak were within the Rossdale Water Treatment Plant service area. The post-disinfectant at Rossdale was chloramine which has a relatively low oxidation potential. Lippy (1984) concluded that the short retention time at Rossdale limited the ability of the chloramine to deactivate *G. lamblia* cysts. The decreasing incidence of giardiasis with increasing distance from Rossdale was consistent with the longer contact time provided the chloramines within the distribution system. In the Edmonton outbreak, although there was no direct evidence to implicate the water supply, no other common source of infection was identified (McClure and Mackenzie, 1988).

Modern water treatment systems are based on the multi-barrier protection provided by coagulation, flocculation, sedimentation, filtration and disinfection (Braidech and Karlin, 1985; Logsdon and Hoff, 1986). Craun (1990) notes that of 95 recognized outbreaks of waterborne giardiasis in the United States during the period 1965–85, 12 involved surface water with no treatment and 40 involved surface water with chlorination only. Many of these outbreaks involved small public or semi-public systems in mountainous areas. These areas often have sparse population and cold, low turbidity surface waters that traditionally have been assumed to be of good quality. However, cold water increases

Table 2.3 Selected outbreaks of waterborne giardiasis in North America

Location	Year	Cases	Treatment Deficiency	Reference
Rome, New York	1974-1975	5000*	Surface water supply could have been contaminated; chloramination only treatment.	Shaw *et al.*, 1977
Camas, Washington	1976	600*	Surface and well water sources; treatment included mixed-media pressure filter and chlorination; filter had ineffective chemical pretreatment; chlorination system failed prior to outbreak.	Kirner *et al.*, 1978; Dykes *et al.*, 1980.
Berlin, New Hampshire	1977	750*	Treatment of river water with pressure filters and chlorination; numerous deficiencies in the condition and operation of filters; common wall separating filtered and unfiltered water leaked.	Lippy, 1978
Banff, Alberta	1982	121	Surface water supply could have been contaminated; chlorination only treatment.	Wilson *et al.*, 1982
Edmonton, Alberta	1982-1983	895	Conventional treatment with chloramine post-disinfectant; softening procedures were not being carried out during the incident.	Lippy, 1984

*estimated

the life span of *Giardia* cysts and slows the reaction rate of some disinfectants (Hibler *et al.*, 1987). Information on the engineering aspects of waterborne giardiasis outbreak investigations and on the ability of specific water treatment practices to remove *G. lamblia* cysts is available elsewhere (Lippy, 1986; Ongerth *et al.*, 1988).

2.2.3 Symptomatology and Diagnosis

The prepatent period for giardiasis was determined to average 9.1 days using human volunteers (Rendtorff, 1954); however, subsequent work has shown that the incubation period for giardiasis is more typically 12 to 19 days (Wolfe, 1990). It is not yet known why the infection is asymptomatic in many individuals, and in others the symptoms range from mild to severe. Up to 60 to 80 percent of infected children in day-care centers are asymptomatic (Wolfe, 1990). In symptomatic infections there is usually an acute stage followed by subacute and chronic stages. The acute stage usually begins with uneasiness in the upper small intestine, with or without low-grade fever, chills, headache, nausea and anorexia. Then there is sudden diarrhea, with explosive, watery, foul-smelling stools, flatulence and abdominal distention (Wolfe, 1990). The acute stage may last only a few days, with the parasite disappearing spontaneously from the host, or it may last for months, leading to malabsorption, chronic diarrhea, steatorrhea, abdominal cramps, weight loss and malnutrition. Commonly the acute stage is followed by subacute and chronic stages, which are characterized by more general abdominal pain, belching, intermittent episodes of soft foul-smelling stools, lassitude, malaise, and occasional episodes of constipation between attacks (Wolfe, 1990).

The routine diagnostic method for giardiasis is the identification of cysts in the feces. This may be difficult due to intermittent excretion, and it is recommended that 3 different specimens taken over a one-week period be examined parasitologically (Meyer and Jarroll, 1980). Exceptionally, the feces can be examined for the presence of trophozoites, or intestinal endoscopy, intestinal intubation or biopsy can be used (Wolfe, 1990). Serological tests for giardiasis are of limited usefulness.

Table 2.4 Drugs used in treatment of giardiasis in North America (Davidson, 1990)

Drug	Origin	Mode of Action	Side Effects
Quinacrine (Atabrine®)	Acridine-yellow dye; initially developed as an anti-malarial.	Intercalating; incorporated into DNA.	Frequently nausea, vomiting, dizziness, headache. In large doses yellow skin discoloration or rarely toxic psychosis.
Metronidazole (Flagyl®)	Imidazole derivative.	Cytotoxic compounds result when exposed to nitroreductase in *Giarida*; disrupts DNA.	Frequently nausea, headache, metallic taste. Occasionally vomiting, diarrhea, vertigo. Disulfiram-like reaction with alcohol.
Furazolidone (Furoxone®)	Synthetic nitrofuran.	Unknown; possibly forms superoxides and other toxic radicals.	Frequently nausea, vomiting, diarrhea, malaise. Disulfiram-like reaction with alcohol.
Paromomycin (Humatin®)	Aminoglycoside.	Unknown.	Frequently nausea, diarrhea, increased gastrointestinal motility. Occasionally headache, vomiting, vertigo.

Eosinophilia in *Giardia*-infected patients is rare. Concomitant eosinophilia and intestinal symptoms are more likely due to infecting hookworm or amebiasis (Wolfe, 1990). An eight-week parasitological follow-up is recommended to confirm the treatment of giardiasis.

2.2.4 Treatment

The drugs most commonly used in North America for treatment of giardiasis are summarized in Table 2.4. Quinacrine has been reported to be 93 percent effective, metronidazole 92 percent effective and furazolidone 84 percent effective (Davidson, 1990). The antimicrobial activity of metronidazole is restricted to anaerobic protozoa such as *Giardia* and *Entamoeba*. Metronidazole is not approved for treatment of giardiasis by the U.S. Food and Drug Administration because it is mutagenic in Ames *Salmonella* bacteria and has been shown to cause tumors in rodents. However, metronidazole is not mutagenic against mammalian cells *in vitro*, and extrapolation of rodent dosages to humans suggests that a "safe" dose is 10 times the adult recommended dosage (Davidson, 1990). Furazolidone is the preferred drug for children because it can be administered in liquid form. Paromomycin has been suggested for use in cases where the patient is pregnant, however more research is required before the safety of this drug is confirmed. It has minimal toxic effect on the patient and fetus because it is poorly absorbed. Additional drug therapies include metronidazole derivatives such as tinidazole (Fasigyn®) and ornidazole (Tiberal®), which are frequently prescribed in Europe and Asia (Davidson, 1990).

2.2.5 Risk Assessment

The basic motivation behind the SWTR is improved public health. In an attempt to provide a rational basis to the SWTR, the USEPA performed an economic analysis of projected costs and perceived benefits which included an analysis of the risk of giardiasis (USEPA, 1989). Estimation of the probability of giardiasis requires consideration of both exposure and dose-response, as indicated in Figure 2.5. The likelihood of illness resulting from infection depends on a number of

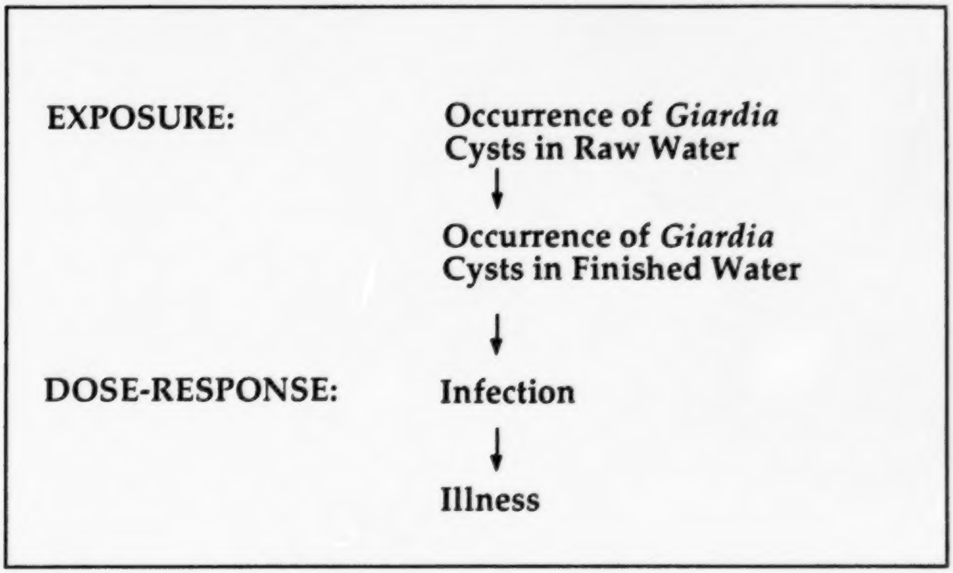

EXPOSURE:
Occurrence of *Giardia* Cysts in Raw Water
Occurrence of *Giardia* Cysts in Finished Water
DOSE-RESPONSE:
Infection
Illness

Figure 2.5 Steps in giardiasis risk assessment

factors including the virulence of the strain of *Giardia* and the immune response of the host (Regli *et al.*, 1991). According to the USEPA, populations should not be subjected to an annual risk of infection from *Giardia* and enteric viruses in public water supplies greater than 1:10,000 or 10^{-4} (USEPA, 1989). In the U.S. surface water sources are used by an estimated 155 million people in 9,801 community and non-community water systems, 29 percent of which provide unfiltered drinking water to some 21 million people (Craun, 1990; USEPA, 1989). By requiring a 99.9 percent reduction or inactivation of *Giardia*, the average annual risk of giardiasis will be reduced from 3×10^{-3} to 6.57×10^{-5} (USEPA, 1989).

A single-hit exponential model has been used to evaluate the health risk of drinking water containing low levels of *Giardia* cysts (Rose and Gerba, 1991; Rose *et al.*, 1991). If the distribution of cysts is random (Poisson), a single cyst is sufficient to cause an infection, and host-microorganism interactions remain constant, the probability of infection (P_i) resulting from the ingestion of a single volume of water (2 L) containing an average number of cysts (N) can be defined as:

$$P_i = 1 - \exp(-rN) \qquad [2.1]$$

where r is the "host-microorganism interaction probability", calculated as -0.02 using the Rendtorff data. The daily risk from equation [2.1] was converted to an annual risk for various levels of occurrence of *Giardia* cysts in source waters, and the effect of treatment reductions determined. To ensure an annual risk of less than 10^{-4}, systems using source waters with geometric concentrations of 0.7, 7.0 and 70 *Giardia* cysts per 100 L would require treatment reductions of 10^{-3}, 10^{-4}, and 10^{-5}, respectively (USEPA, 1989; Rose *et al.*, 1991). Thus, in highly polluted source waters more than the 99.9 percent reduction or inactivation of *Giardia* required by the SWTR may be necessary to keep the annual risk below 10^{-4}. These source waters would typically require filtration.

The risk of infection from equation [2.1] may be overestimated based on the assumptions that all cysts are viable, a single cyst is sufficient to cause an infection in humans and the daily consumption of tap water is 2 L. Peak contamination levels and the duration of exposure are not taken into account using the exponential model, however, which would tend to underestimate risk.

The host-microorganism interaction probability in equation [2.1] was calculated using the Rendtorff (1954) data. In the Rendtorff data the sample size was 2 for the 10 cyst dose (both subjects positive), 20 for the 25 cyst dose (30 percent of subjects positive) and 2 for the 100 cyst dose (both subjects positive). No other human feeding experiment data are available. Schaefer *et al.* (1991) determined the *G. lamblia* infective dose for the Mongolian gerbil using cohorts of ten animals and serial dilutions. Their results were 1 of 10 gerbils positive at the 10 cyst dose; 4 of 10 gerbils positive at the 80 cyst dose; and 10 of 10 gerbils positive at the 10,000 cyst dose. The 25 cyst dose response from the Rendtorff (1954) data is similar to that in the Schaefer *et al.* (1991) data, but the other two responses are not.

Analyzing health and environmental risks is a new field. The distribution of cysts should be based on monitoring data and may be found to follow other distributions such as lognormal or negative binomial (Ongerth, 1989; Regli *et al.*, 1991). Available dose-response models include the beta-Poisson "infectivity probability", beta-negative binomial and lognormal models (Haas, 1983; Regli *et al.*, 1991).

2.3 OZONE CHEMISTRY

Two primary oxidation reactions for ozone in water have been proposed (Hoigné and Bader, 1975):

1. Direct oxidation which is a selective oxidation of compounds by the ozone molecule. These reactions can take several minutes and result in non-reactive oxidated species. In the absence of ozone decomposition, the ozone concentration can remain relatively constant over short time intervals; and

2. Oxidation by radical intermediates where the hydroxyl radical is believed to be the most important of the ozone decomposition products. It is highly reactive and has a life span of only a few microseconds in water.

The direct reaction of molecular ozone (O_3) with solute M is:

$$O_3 + M \xrightarrow{k_{d,M}} M_{\text{oxidized}} \qquad [2.2]$$

where: $k_{d,M}$ is the direct reaction rate constant of [M].

This reaction is first-order with respect to the concentrations of both ozone and M, and the rates of oxidation of M and consumption of ozone can be described as (Hoigné, 1982):

$$-\frac{d\,[M]}{dt} = -\eta\,\frac{d\,[O_3]}{dt} = k_{d,M}\,[M]\,[O_3] \qquad [2.3]$$

where: η is a stoichiometric factor.

2.3.1 Properties of Ozone

Ozone is an extremely oxidative, unstable, corrosive gas that must be generated on-site (IOA, 1990). Factors which affect the chemistry of ozone in water include its solubility, decomposition, temperature and pH (Rice, 1989). The electrochemical generation of ozone at 25°C can be written as (Bablon *et al.*, 1991a):

$$H_2O + O_2 \rightarrow (O_3)\,(\text{aqueous}) + 2\,H^+ + 2\,e^- \quad E^o = +\,2.07\ V \qquad [2.4]$$

Molecular ozone has a dipolar structure which can lead to cyclo addition on unsaturated bonds. The ozone molecule can also act as an electrophilic agent or as a nucleophilic agent (Bablon *et al.*, 1991a). Thus, ozone is able to oxidize carbon-carbon double bonds, aromatic compounds, and most low oxidation state nitrogenous and sulfurous compounds (United States National Research Council, 1980). However, it is the kinetics of reactions that control the oxidizing ability of ozone in water treatment. The second-order rate constant for ozone can vary by more than eight orders of magnitude ranging from 10^7 $M^{-1}s^{-1}$ for the phenolate anion to 10^{-1} $M^{-1}s^{-1}$ for tetrachloroethylene (Glaze, 1986). The direct reaction of ozone is therefore highly selective.

Ozone is more soluble than oxygen in water but because of much lower partial pressures it is difficult to obtain concentrations of more than a few milligrams per liter under normal conditions of temperature and pressure (Venosa, 1972). The solubility of ozone is governed by Henry's law, which states that the solubility of a gas in water is proportional to its partial pressure in the gas phase. The greater the Henry's constant, the less soluble the gas. If gas phase ozone is 3 percent (wt), ozone solubility in water is 10.58 mg/L at 25°C and 22.18 mg/L at 5°C (Rice, 1989). Ozone generators typically produce 1–3 percent (wt) ozone when air is used as the feed gas and 4–6 percent when pure oxygen is used (Bellamy *et al.*, 1991). As with oxygen, ozone solubility increases with decreasing temperature; however, the temperature dependence of Henry's law constant is 1.74 times greater for ozone (James M. Montgomery Consulting Engineers, 1985). Henry's law constant for the ozone-water system has been experimentally determined as a function of temperature, pH and ionic strength in the presence of various salts (Sotelo *et al.*, 1989).

Ozone has long been recognized as an air pollutant and is named after the Greek *ozein*, meaning "to smell", for its distinctive odor, which is detectable at concentrations as low as 0.01–0.05 ppm (Rice *et al.*, 1981). A 60 min exposure of 10 ppm (vol) ozone would cause temporary toxicity to humans; concentrations of 100 ppm and 1,000 ppm for 60 min would cause permanent toxicity and death, respectively (IOA, 1990). Ozone gas has been shown to cause adverse effects to the eye and nervous system and has effects on the lung ranging from alveolar damage to pulmonary edema (Bablon *et al.*, 1991a). Less is known of the toxicological effects of aqueous ozone on mammals; however, the effects have been well studied in several fish species. The rainbow trout is very sensitive to ozone. Ozone concentrations must be less than 0.002 mg/L to avoid significant effects upon exposures of more than three months (Bablon *et al.*, 1991a). In drawing conclusions on aqueous ozone toxicity, the simultaneous exposure to aqueous ozone and ozone reaction by-products must be considered.

2.3.2 Decomposition of Ozone

Factors influencing the decomposition of molecular ozone in water include pH, temperature, the organic and inorganic constituents of the water and the presence of organic and inorganic initiators (Hoigné and Bader, 1979a; Bablon *et al.*, 1991a). However, the presence of radical scavengers such as carbonate and bicarbonate ions can reduce the rate of decomposition of ozone by quenching the chain reaction (Doré *et al.*, 1987; Hoigné, 1982; Glaze, 1986). Temperature has opposing influences on ozone stability and disinfection efficiency. Increased temperature reduces the solubility and stability of ozone in water, but increases the reaction rate between microorganisms and ozone in accordance with van't Hoff–Arrhenius theory (Bablon *et al.*, 1991b). It has been reported that the increased reaction rate effect exceeds that of ozone instability (Farooq *et al.*, 1977; Roy *et al.*, 1982). Low pH favors direct reactions with ozone while high pH promotes the autodecomposition of ozone (James M. Montgomery Consulting Engineers, 1985). However, at pH values greater than 10.3, the carbonate ion is the dominant species. The reaction rate constant for the hydroxyl radical with the carbonate ion is over 20 times greater than with the bicarbonate ion (Glaze *et al.*, 1987).

Over the past 15 years, Hoigné and coworkers have studied ozone decomposition in pure and natural surface waters and have measured rate constants for ozone reactions with many organic and inorganic compounds in water (Hoigné and Bader, 1975; 1978; 1979a; 1979b; Hoigné, 1982). The aqueous ozone decomposition process is an autocatalytic chain-reaction sequence in which decomposition products catalyse further depletion of molecular ozone (Grasso and Weber, 1989). Two models for aqueous ozone decomposition dominate those that have been proposed: the Staehelin, Bühler and Hoigné (SBH) model and the Tomiyasu, Fukutomi and Gordon (TFG) model (Bablon *et al.*, 1991a; Grasso and Weber, 1989; Staehelin and Hoigné, 1985; Tomiyasu *et al.*, 1985). The main differences between the two models were summarized by Chelkowska *et al.* (1992) as:

(i) The initiation step in the SBH mechanism is characterized formally by an oxygen cation radical (O^+) transfer from the ozone to the hydroxide ion, while the TFG initiation step corresponds to an oxygen atom transfer between the same reactants.

(ii) The SBH mechanism assumes the formation of two intermediate species - HO_3 and HO_4 - which are not included in the TFG mechanism.

(iii) According to the SBH mechanism, the ozonide ion radical (O_3^-) is considered in the propagation reactions only as the precursor of the HO_3 radical. In the TFG model O_3^- is one of the most important chain-carriers, which participates in different electron and atom transfer steps.

Table 2.5 presents a simplified version of the postulated reaction mechanisms for the two models. More complete sets of reactions including original and modified rate constants can be found elsewhere (Chelkowska *et al.*, 1992). Of the species shown in Table 2.5, the most reactive is the hydroxyl free radical, having an oxidation potential of $E^0 = +2.80$ V (Rice and Gomez-Taylor, 1987). The SBH model with its originally proposed rate constants generally predicts a faster ozone decomposition than that observed experimentally in the basic pH range (Chelkowska *et al.*, 1992). Neither model adequately describes ozone decomposition in natural waters.

Ozone decomposition can be divided into three steps: initiation, propagation and termination (Figure 2.6). In pure water the initiator is typically the hydroxide ion and hundreds of ozone molecules can be decomposed in the propagation step. Other initiators include the hydroperoxide ion (hydrogen peroxide), ferrous iron, natural organics such as humic substances, formic acid, u.v. light and microorganisms (Bablon *et al.*, 1991a). Initiation reactions are a function of pH. There is no ozone decomposition at pH 2 in phosphate buffer (Hoigné and Bader, 1978; 1979b). Promoters are all organic and inorganic molecules capable of regenerating the superoxide anion from the hydroxyl radical and include phosphate species, primary alcohols, formic acid, humic acid, and ozone itself in pure waters (Bablon *et al.*, 1991a). The superoxide anion reacts extremely quickly with ozone, promoting its decomposition.

Table 2.5 Postulated reactions mechanisms in ozone decomposition. SBH, Staehelin, Bühler, Hoigné; TFG, Tomiyasu, Fukutomi, Gordon. (after Bablon *et al.*, 1991a)

Model	Reaction	Decomposition Step	Equation
SBH	$O_3 + OH^- \rightarrow HO_2^{\bullet} + {}^{\bullet}O_2^-$	Initiation	[2.5]
	$HO_2^{\bullet} \leftrightarrow H^+ + {}^{\bullet}O_2^-$	Propagation	[2.6]
	${}^{\bullet}O_2^- + O_3 \rightarrow O_2 + {}^{\bullet}O_3^-$	Propagation	[2.7]
	${}^{\bullet}O_3^- + H^+ \leftrightarrow HO_3^{\bullet}$	Propagation	[2.8]
	$HO_3^{\bullet} \rightarrow {}^{\bullet}OH + O_2$	Propagation	[2.9]
	${}^{\bullet}OH + O_3 \rightarrow HO_4^{\bullet}$	Propagation	[2.10]
	$HO_4^{\bullet} \rightarrow HO_2^{\bullet} + O_2$	Propagation	[2.11]
	$HO_4^{\bullet} + HO_4^{\bullet} \rightarrow H_2O_2 + 2O_3$	Termination	[2.12]
	$HO_4^{\bullet} + HO_3^{\bullet} \rightarrow H_2O_2 + O_2 + O_3$	Termination	[2.13]
TFG	$O_3 + OH^- \rightarrow HO_2^- + O_2$	Initiation	[2.14]
	$H_2O_2 \leftrightarrow H^+ + HO_2^-$	Initiation	[2.15]
	$HO_2^- + O_3 \rightarrow HO_2^{\bullet} + {}^{\bullet}O_3^-$	Propagation	[2.16]
	${}^{\bullet}O_2^- + O_3 \rightarrow {}^{\bullet}O_3^- + O_2$	Propagation	[2.17]
	${}^{\bullet}O_3^- + H_2O \rightarrow {}^{\bullet}OH + O_2 + OH^-$	Propagation	[2.18]
	${}^{\bullet}O_3^- + {}^{\bullet}OH \rightarrow {}^{\bullet}O_2^- + HO_2^{\bullet}$	Propagation	[2.19]
	${}^{\bullet}O_3^- + {}^{\bullet}OH \rightarrow O_3 + OH^-$	Propagation	[2.20]
	$HO_2^{\bullet} + OH^- \leftrightarrow H_2O + {}^{\bullet}O_2^-$	Propagation	[2.21]
	${}^{\bullet}OH + O_3 \rightarrow HO_2^{\bullet} + O_2$	Propagation	[2.22]
	${}^{\bullet}OH + CO_3^{-2} \rightarrow OH^- + CO_3^{\bullet -}$	Termination	[2.23]
	$CO_3^{\bullet -} + O_3 \rightarrow CO_2 + {}^{\bullet}O_2^- + O_2$	Termination	[2.24]

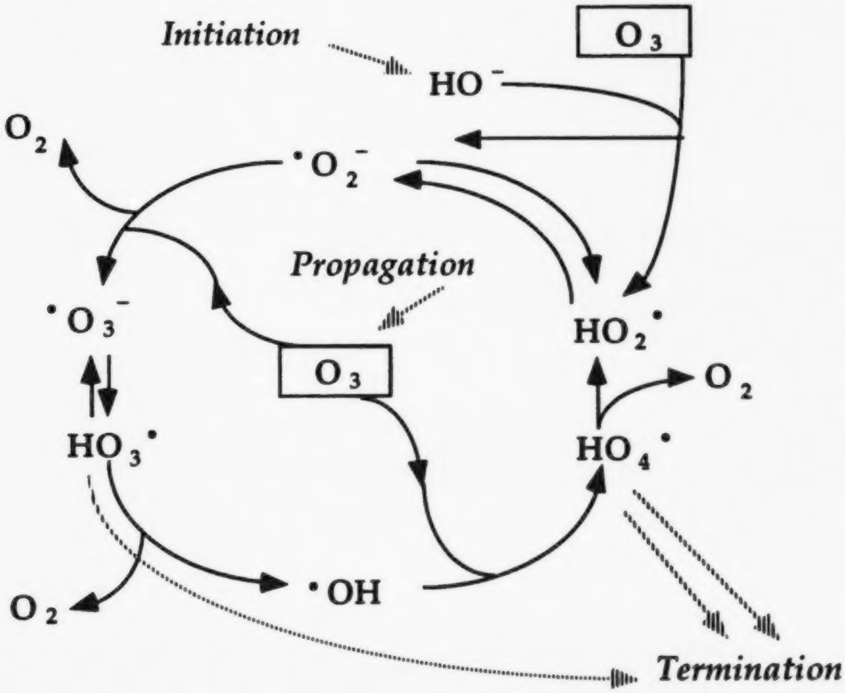

Initiation
HO⁻
O₃
O₂
•O₂⁻
Propagation
•O₃⁻
HO₂•
O₃
O₂
HO₃•
HO₄•
O₂
•OH
Termination

Figure 2.6 Ozone decomposition cycle assuming Staehelin, Bühler and Hoigné mechanism (after Glaze, 1986)

The ozonide anion is also considered an important promoter in the TFG mechanism. Inhibitors of the ozone decomposition cycle include bicarbonate ions, carbonate ions (equations 2.23 and 2.24), alkyl groups, tertiary alcohols such as *tert*-butyl alcohol, and humic substances (Bablon *et al.*, 1991a). Table 2.5 classifies reactions as being initiation, propagation or termination steps in the ozone decomposition cycle.

Ozone has a half-life of approximately 165 min in distilled water (20°C) under quiescent conditions (Rice *et al.*, 1981). The half-life of ozone in water and wastewater engineering applications is typically much less due to the presence of demand causing substances and other losses such as volatilization to the atmosphere. The half-life of ozone, being merely a single point, is of less importance than the entire decomposition kinetics. There have been numerous studies on aqueous ozone decomposition kinetics (Gurol and Singer, 1982; Staehelin and Hoigné, 1985; Tomiyasu *et al.*, 1985; Yurteri and Gurol, 1988; Grasso and Weber, 1989). Gurol and Singer (1982) reviewed the literature on ozone decomposition kinetics and reported that the reaction order relative to ozone concentration ranged from 1 to 2. Differences were attributed to (Gurol and Singer, 1982):

(i) the use of different, sometimes questionable, analytical techniques to measure the concentration of dissolved ozone,
(ii) uncertainties in the data analysis and data interpretation,
(iii) the effect of solution composition, e.g., the ionic medium, on decomposition, and
(iv) the possible presence of impurities in the reagents used.

Most researchers used only an integral method for data analysis. Gurol and Singer (1982) used initial-rate and differential analysis methods in addition to the integral method and determined that the rate of ozone decomposition (r_{O_3}) can be expressed as:

$$r_{O_3} = -\frac{d\,[O_3]}{dt} = k_O\,[OH^-]^a\,[O_3]^b \qquad [2.25]$$

where: k_O is a rate constant specific to the chemical composition of the aqueous system; and

a, b	are the reaction orders with respect to hydroxide ion and ozone concentrations, respectively.

At 20°C and in the pH range 4–10, under both quiescent and dynamic conditions, it was determined that "a" was 0.55, and "b" was 2 (Gurol and Singer, 1982). Below pH 4, decomposition was found to be relatively slow and insensitive to pH.

Tomiyasu *et al.* (1985) empirically fit the following expression to their stopped-flow data:

$$r_{O_3} = -\frac{d\,[O_3]}{dt} = k_1\,[OH^-]\,[O_3] + k_2\,[OH^-]\,[O_3]^2 \qquad [2.26]$$

where:	k_1, k_2	are rate constants that vary markedly with solution composition.

Tomiyasu *et al.* (1985) noted that when the solution pH decreased, or when there were sufficient radical scavengers present, the initiation step became rate limiting, and the mixed-order expression reduced to first-order with respect to ozone concentration.

In natural waters ozone decomposition is accelerated and ozone chemistry becomes even more complex. Pseudo-n-order kinetics have been reported to be inappropriate for ozone decomposition in filtered activated sludge effluents because consumption rates depended on how much ozone had already been consumed, as well as on instantaneous ozone concentration (Watt *et al.*, 1989). A modified rate expression was proposed which was first-order with respect to ozone but in which the rate constant (k) was replaced by a variable called the specific ozone utilization rate (w) (Watt *et al.*, 1989). Natural surface waters contain complex molecules such as humic molecules. Sites that ozone may attack in these molecules include: carbon-carbon double bonds, aromatic rings activated with phenolic groups, and complexed metals such as iron (Glaze, 1986). Collective parameters, including TOC and DOC in surface waters and COD and BOD in wastewaters, are useful in describing ozone decomposition in waters with impurities.

When ozone reacts with substances in natural waters it is very likely reacting in a combination of the direct oxidation and oxidation by radical intermediates pathways (Glaze *et al.*, 1987). Staehelin and Hoigné (1985) expressed total ozone consumption in waters with solutes M_i as:

$$-\frac{d\,[O_3]}{dt} = k_c\,[O_3] + \Sigma(k_{d,i}\,[M_i]\,[O_3]) \qquad [2.27]$$

$$k_c = k_1\,[OH^-] + \{2k_1\,[OH^-] + \Sigma(k_{I,i}\,[M_i])\}(1 + \frac{\Sigma(k_{P,i}\,[M_i])}{\Sigma(k_{S,i}\,[M_i])}) \qquad [2.28]$$

where:	k_c	is the radical cyclic reaction rate constant;
	$k_{d,i}$	is the direct reaction rate constant of $[M_i]$; and
	I, P, S	are individual solutes M that predominantly act by one single pathway; initiator, promoter or scavenger, respectively.

Equation [2.28] was derived using a mass balance about $^{\bullet}O_2^-$ and assuming that all $^{\bullet}O_3^-$ formed decomposed into the hydroxyl radical. If, as in most waters of interest,

$$k_I\,[M] >> 2k_1\,[OH^-] \qquad [2.29]$$

and most of the scavenging of hydroxyl radicals are due to scavengers S, which can be distinguished from M, then:

$$k_c = k_I[M](1 + \frac{(k_P\,[M])}{(k_S\,[S])}) \qquad [2.30]$$

Yurteri and Gurol (1988) simplified the SBH model to obtain an expression for dissolved ozone consumption in natural waters:

$$-\frac{d\,[O_3]}{dt} = w\,[O_3] \qquad [2.31]$$

$$\log w = B_O + a(pH) + b\,\{\log(TOC)\} - c\{\log(\frac{alkalinity}{10})\} \qquad [2.32]$$

where:	B_O, a, b, c	are experimentally determined coefficients;

w	is the specific ozone utilization rate in h^{-1}; and
alkalinity	is a measure of bicarbonate and carbonate ion concentration in mg/L as $CaCO_3$.

A total of 96 runs in synthetic waters at pH 6.8–9.0; TOC 0.3–5.3 mg/L; and alkalinity of 10–500 mg/L as $CaCO_3$ were conducted at 20 ± 1 °C to obtain the following values for the coefficients (Yurteri and Gurol, 1988):

$$\log w = -3.98 + 0.66(\mathrm{pH}) + 0.61\{\log(\mathrm{TOC})\} - 0.42\{\log(\frac{\mathrm{alkalinity}}{10})\} \qquad [2.33]$$

The three independent variables accounted for 83 percent of the observed variation in w. When further runs were done using 11 natural waters with pH 6.0–7.1; TOC 1.7–7.3 mg/L; and alkalinity of 15–90 mg/L as $CaCO_3$, it was possible to predict w to ± 25 percent using equation [2.33]. Typical values for w were 5 h^{-1} for groundwaters and 50 h^{-1} for contaminated surface waters; however, no data was presented for ozone consumption in pure waters (Yurteri and Gurol, 1988).

Ozone decomposition follows Arrhenius-type behavior (James M. Montgomery Consulting Engineers, 1985; Roth and Sullivan, 1983):

$$r_{O_3} = -\frac{d\,[O_3]}{dt} = k_o\,\{\exp\,[\,-\frac{E}{R}(\frac{1}{T})]\}\,[OH^-]^a\,[\,O_3\,]^b \qquad [2.34]$$

where:	E	is the Arrhenius activation energy in cal/mole;
	R	is the ideal gas law constant (1.987 cal/mole °K);
	T	is the absolute temperature in °K; and
	k_o, a, b	are constants.

The Arrhenius activation energy for ozone assuming first-order decomposition kinetics has been reported to be 7.79–15.3 kcal/g-mole (Roth and Sullivan, 1983).

2.3.3 Oxidation Competition Value

The oxidation competition value (Ω_M) is defined as the amount of decomposed ozone required to reduce the concentration of a substrate solute to 37 percent of its initial value in a given water (Hoigné and Bader, 1979b; Hoigné, 1982; Bancroft *et al.*, 1984). The motivation behind the development of Ω_M was to determine (Hoigné and Bader, 1979b):

(i) the spontaneous ozone requirement,
(ii) the lifetime of the ozone, and
(iii) the oxidation efficiency of the secondary oxidants derived from the decomposed ozone (hydroxyl radicals) in different waters.

Mathematically, provided $k_M \times [M] << \Sigma(k_i[S_i])$,

$$-\frac{d[M]}{dt} = \eta \frac{d(\Delta O_3)}{dt} \times \frac{k_M \times [M]}{\Sigma(k_i[S_i])} \qquad [2.35]$$

where:

$[M]$	is the concentration of the solute micropollutant M;
ΔO_3	is the amount of decomposed ozone;
η	is the hydroxyl radical yield per mole of ΔO_3;
k_M	is the reaction rate constant of [M];
S_i	is the radical scavenging solute i; and
k_i	is the reaction rate constant of S_i.

Defining

$$\Omega_M = \frac{\Sigma(k_i[S_i])}{\eta\, k_M} \qquad [2.36]$$

and substituting into equation [2.35],

$$-\frac{d[M]}{dt} = \frac{d(\Delta O_3)}{dt} \times \frac{[M]}{\Omega_M} \qquad [2.37]$$

Rearranging and integrating equation [2.37],

$$\Omega_M = \frac{-\Delta O_3}{\ln([M]_\infty/[M]_o)} \qquad [2.38]$$

where $\ln([M]_\infty/[M]_o)$ is the relative concentration of micropollutant M remaining after a given amount of ozone has decomposed. When [M] is 37 percent of its initial value, $\Omega_M = \Delta O_3$.

It is useful to define the oxidation competition coefficient

$$\omega_{M,i} = \frac{1}{\eta}\frac{k_i}{k_M} \qquad [2.39]$$

Then Ω_M can be expressed as the linear sum of all impurities which consume hydroxyl radicals,

$$\Omega_M = \Sigma(\omega_{M,i}\,[S_i]) \qquad [2.40]$$

If either Ω_M or ω_M are known for micropollutant A (Ω_A,ω_A), then the corresponding values for micropollutant B (Ω_B,ω_B) can be readily calculated since Ω_B/Ω_A or $\omega_B/\omega_A = k_A/k_B$ (Hoigné and Bader, 1979b). This methodology was used to determine the reaction rate constants between ozone and a variety of organic and inorganic compounds of interest in environmental engineering (Hoigné, 1982).

Ω_M ranged from 0.3 mg/L for a soft groundwater to 2.2 mg/L for a highly eutrophic lake in an experimental study using benzene as a reference solute in 40 different Swiss waters (Hoigné and Bader, 1979b). It was found that Ω_M increased 0.25 mg/L per mg/L dissolved organic carbon present in the Swiss waters. Knowledge of Ω_M, which relates to hydroxyl radical consumption, has implications regarding ozone reactions which are competitive against direct reactions. Studies generally support direct oxidation reactions as being more important in disinfection (Singer, 1990).

2.3.4 Advanced Oxidation Processes

Advanced oxidation processes (AOPs) are essentially engineered chemical systems in which one oxidant is catalyzed by another or u.v. radiation to take advantage of radical reaction pathways (Glaze *et al.* 1987). The most common processes are ozone–hydrogen peroxide, ozone–u.v., hydrogen peroxide–u.v. and ozone–hydroxyl ions or ozonation at elevated pH. The mechanisms of AOPs are not fully understood. Researchers with the MWDSC have named the ozone–hydrogen peroxide process PEROXONE (Ferguson *et al.*, 1990). Ozone–hydrogen peroxide has been used in trihalomethane formation potential (THMFP) control, oxidation of toxic compounds in surface and groundwaters and in the control of compounds associated with taste and odors (Glaze *et al.*, 1987; Ferguson *et al.*, 1990; Glaze *et al.*, 1990). There is limited evidence that hydrogen peroxide in very high concentrations in drinking water (4,000 mg/L) can induce tumors in mice (Bull and Kopfler, 1991). Mammals generate large amounts of hydrogen peroxide endogenously; however, it is likely that the carcinogenic activity of hydrogen peroxide depends on its conversion to the reactive hydroxyl radical. This occurs in the presence of free iron in accordance with the Fenton reaction (Fenton, 1894).

Hydrogen peroxide accelerates the rate of ozone decomposition, increasing the quantity of high-energy, short-lived hydroxyl radicals (Wolfe *et al.*, 1989a). When combined with water, hydrogen peroxide partially dissociates into the hydroperoxide ion, which can initiate the ozone decomposition cycle as follows (Bablon *et al.*, 1991a):

$$H_2O_2 + H_2O \leftrightarrow HO_2^- + H_3O^+ \qquad k_a=10^{-11.6} \qquad [2.41]$$

$$O_3 + HO_2^- \leftrightarrow OH^\bullet + {}^\bullet O_2^- + O_2 \qquad [2.42]$$

Hydrogen peroxide reacts very slowly with ozone; however, the hydroperoxide anion is highly reactive. Therefore the rate of ozone decomposition by hydrogen peroxide accelerates with increasing pH. At about pH 10 self-destruction of hydroxyl radicals by excess hydrogen peroxide occurs (Brunet *et al.*, 1984):

$$OH^{\bullet} + H_2O_2 \leftrightarrow H_2O + HO_2^{\bullet} \qquad [2.43]$$

$$OH^{\bullet} + HO_2^{\bullet} \leftrightarrow O_2 + H_2O \qquad [2.44]$$

The optimal condition for oxidizing tetrachloroethylene was reported to be at a hydrogen peroxide to ozone weight ratio of 0.7–0.9 while that for geosmin was at a weight ratio of 0.2–0.3 (Glaze *et al.* 1987; Ferguson *et al.*, 1990). Thus, the controlling factors for the oxidative destruction of different types of organic substrates appear to be pH and the hydrogen peroxide to ozone weight ratio.

Little is known regarding the effectiveness of ozone–hydrogen peroxide in disinfection (Singer, 1990). Duguet *et al.* (1989) studied *Bacillus cereus* inactivation using hydrogen peroxide to ozone weight ratios of 0.4–1.0. They concluded that ozone–hydrogen peroxide was applicable for disinfection but was approximately 1.5 log less efficient than ozonation alone. Other work has also suggested that the more active bactericidal agent in the ozone–hydrogen peroxide process is the ozone molecule rather than the hydroxyl radical (Wolfe *et al.*, 1989a; Wolfe *et al.*, 1989b; Yuen, 1990). Hydrogen peroxide alone has been reported to be an ineffective disinfectant. The U.S. National Research Council (1980) reported that 90 percent inactivation of *E. coli* required contact times in excess of 300 min and hydrogen peroxide concentrations of 30–90 mg/L.

2.4 OZONE DISINFECTION OF WATER

2.4.1 Mode of Action

There is little agreement in the literature regarding the mode of action of ozone on microorganisms. Some studies using bacteria reported that ozone alters proteins and fatty acid double bonds in the cell membrane, adversely affecting permeability (Scott and Lesher, 1963; Pryor *et al.*, 1983). Other studies reported that ozone may affect DNA in the cell, causing cell inactivation (Hamelin and Chung, 1974; Ohlrogge and Kernan, 1983; Ishizaki *et al.*, 1987). *In vivo* induction of the antioxidant enzymes, catalase and superoxide dismutase, was observed in *E. coli* for 30 min after ozone exposure (Whiteside and Hassan, 1987). These enzymes were inactivated in cell-free extracts and in cells exposed to ozone. However, loss of activity was more pronounced in the cell-free extracts due to their lack of induction capabilities. This may represent an adaptive response to defend cells against ozone toxicity (Whiteside and Hassan, 1987).

Little information is available on the mode of action of ozone on protozoa. The initial lag in *G. muris* cyst inactivation kinetics was speculated to be due to the time dependent nature of ozone penetration through the cyst wall and membrane (Wickramanayake, 1984). The observation of leached cytoplasm from *G. duodenalis* cysts led to speculation that ozone caused a progressive breakdown of the cyst wall, plasma membrane and cytoplasm (Wallis *et al.*, 1990). Others have speculated that ozone causes cyst lysis (J. Ongerth, Univ. of Washington, Seattle, Wash., 1990, Personal communication). Until greater knowledge of the chemical structure of cyst walls permits generalization, mode of action data must be regarded as organism specific.

2.4.2 Chemical Reaction Pathway

An unresolved issue is whether direct oxidation or oxidation by radical intermediates predominate in disinfection reactions. Most studies support direct oxidation reactions as being more important in disinfection; however, research on this topic is ongoing (Singer, 1990). Hoigné and Bader (1975; 1978) stated that disinfection was the result of direct ozone oxidation. They noted that the reactions of hydroxyl radicals with molecularly dissolved substrates were often so fast (microseconds) that the radicals were consumed before they encountered a microorganism. However, they also noted that, depending on the solutes present, the hydroxyl radical may form intermediate products which may play a part in disinfection. The work of Bancroft *et al.* (1984) suggested a hydroxyl radical mediated mechanism for disinfection based on the correlation observed between inactivation and oxidation competition value and the fact that Ω relates to the amount of hydroxyl radical material present in the water and does not give information on direct ozone reactions (Hoigné and Bader, 1979b). Oxidation by hydroxyl radicals was also suggested by Dahi (1976); however, some of the effects he observed may have been due to synergism between ultrasound and ozone. Studies which reported that pH had minimal effect on ozone disinfection of water support a direct ozone reaction (Venosa, 1972; Ross *et al.*, 1976).

A pilot-scale investigation of the disinfection efficacy of ozone–hydrogen peroxide and ozone in two surface waters found that ozone–hydrogen peroxide was less efficient at higher hydrogen peroxide to ozone weight ratios and this was postulated to be due to a concomitant lower ozone residual (Wolfe *et al.*, 1989a; Wolfe *et al.*, 1989b). Other studies using *E. coli* and *B. cereus* in laboratory waters reported that ozone–hydrogen peroxide adversely affected disinfection performance compared to ozonation alone (Duguet *et al.* 1989; Yuen, 1990). These studies reported that the direct ozone reaction was probably more important in disinfection.

2.4.3 Kinetic Modelling of Disinfection

Modelling the chemical disinfection of water began in 1907–08 with the historic work of H. Chick, and Madsen and Nyman (Chick, 1908; Madsen and Nyman, 1907). These researchers worked simultaneously but independently on the disinfection of anthrax spores with phenol and mercuric chloride, respectively, and discovered that the concentration of spores remaining alive varied logarithmically with time (Chick, 1908). Chick (1908) postulated that the disinfection reaction rate was dependent on the relative concentrations of spores and disinfectant. If the disinfectant concentration was constant, the reaction would be first-order with respect to surviving spores. Watson (1908), using Chick's data, expanded the model to account for changes in disinfectant concentration. The Chick-Watson model is still the main disinfection theory in use today. It may be expressed as (Haas and Karra, 1984a):

$$\log \frac{N}{N_o} = -kC^n t \qquad [2.45]$$

where:	N_o	is the initial concentration of microorganisms;
	N	is the concentration of surviving microorganisms at time t;
	k	is the pseudo first-order reaction rate constant;
	C	is the residual concentration of disinfectant; and
	n	is the coefficient of dilution, an empirical factor.

If n is greater than one, disinfectant concentration is the dominant factor in determining the inactivation rate while if n is less than one, exposure time is more important than disinfectant concentration. The Chick-Watson model has been reported to be adequate for describing chlorine inactivation kinetics in chlorine demand-free waters for a variety of microorganisms including bacteria, viruses and *Naegleria* (Haas and Karra, 1984a).

The Ct concept is a simplification of equation 2.45 that assumes n equals one. This results in the expression "Ct = constant" for a particular microorganism and specified kill at a given temperature and pH. Hoff (1987) summarized strengths and weaknesses of the Ct concept. The main advantage of using Ct values was that they are based on experimental data with a concomitant disadvantage in the limited size of the experimental database (Hoff, 1987). However, Ct is only a portion of the mathematics of kinetic expressions and does not adequately describe macromixing phenomenon including residence time distribution, micromixing and other nonidealities such as temperature and water quality (Severin and Churn, 1987). These authors have based their conclusions mainly on data from chlorine disinfection experiments.

Prokop and Humphrey (1970) reviewed various empirical, mechanistic, and stochastic models which have been formulated to describe disinfection kinetics and deviations from the Chick-Watson model. Models postulated since this review include turbulent reactive-flow models (Haas, 1981), series-event models (Severin *et al.*, 1984) and a multi-Poisson distribution model (Wei and Chang, 1975). Hom (1972) proposed the following variation of the Chick-Watson model:

$$\log \frac{N}{N_0} = -kC^n t^m \qquad [2.46]$$

where: m is the time coefficient, and other parameters are as described for equation 2.45.

The Hom model can be used to describe variable inactivation rates. If m is greater than one, the inactivation rate increases with time while if m is less than one, the inactivation rate decreases with time.

The Selleck model has been used to describe an initial lag as well as a declining inactivation rate (James M. Montgomery Consulting Engineers, 1985; Haas and Heller, 1990):

$$\log \frac{N}{N_0} = -n \log (1 + bCt) \qquad [2.47]$$

where: n, b are empirical constants, and other parameters are as described for equation 2.45.

The Selleck model is appropriate in describing the chlorine inactivation kinetics of *E. coli* in wastewater and other systems containing chlorine demand (Haas and Karra, 1984b).

In the Chick-Watson, Hom and Selleck models disinfection is modelled as a function of three variables: the concentration of the disinfectant, the number of microorganisms, and contact time.

2.4.4 Ozone Inactivation Kinetics

Various shapes of ozone inactivation curves have been reported in the literature. For example, investigators using poliovirus type 1, coliphage T_2, *B. cereus* or *E. coli* found that ozone inactivation had two stages; a rapid initial stage, followed by a much slower second stage (Katzenelson *et al.*, 1974; Finch *et al.*, 1988; Duguet *et al.*, 1989). A three stage inactivation curve was reported in a study using *E. coli*, ultrasound and ozonation (Dahi, 1976). In this case the initial ozone demand stage was interpreted as being prior to the establishment of a proper free radical activity (Dahi, 1976). The ozone inactivation of *E. coli* has also been reported to be a continuous function, though with a rapidly decreasing rate constant (Yuen, 1990).

Originally the sigmoidal or upward concave nature of survival curves was explained in terms of a vitalistic concept (Cerf, 1977). It was postulated that survival times should be normally distributed because the majority of microorganisms in a homogeneous population possess an average degree of resistance, while a minority possess a greater or lesser degree of resistance (Cerf, 1977). Survival curves were plotted on probability paper with a logarithmic time scale (log-probit plots). However, Roy *et al.* (1981) and other investigators demonstrated that the vitalistic concept could not adequately explain the nonlinear nature of survival curves in carefully designed experiments, and a mechanistic concept has subsequently become generally accepted. According to the mechanistic concept (Cerf, 1977; Roy *et al.*, 1981):

1. the destruction of microorganisms is an orderly process presenting a close analogy to chemical reactions;
2. nonlinear survival curves may be obtained if the reaction order is more than one;
3. nonlinear survival curves may be obtained if the mechanism follows a series of first-order unimolecular reactions;
4. nonlinear survival curves may be obtained if the mechanism consists of a number of concurrent first-order reactions on different sites of the microorganism; and
5. nonlinear survival curves might be an artifact independent of the mechanism of inactivation. Artifacts include genetic heterogeneity, heterogeneity of treatment (local variations of temperature or of disinfectant concentration), clumping and difficulties in the counting of low levels of survivors.

Survival curves plotted as log survival versus time, as is current practice (semi-log plots), implicitly accept the mechanistic concept. Numerous researchers have attributed nonlinearity, specifically a two-stage kinetic phenomena, to heterogeneous populations with varying resistances to the disinfectant (Prokop and Humphrey, 1970; Hom, 1972; Bancroft *et al.* 1984). Clumping has also frequently been postulated as the cause of deviations from first-order kinetics in ozone disinfection data (Katzenelson *et al.*, 1974; Dahi, 1976). The physiological status of the test microorganism can influence inactivation kinetics and give large underestimates of the Ct required to achieve inactivation (Berg, 1987). Scott and Lesher (1963) found that *E. coli* were more sensitive to ozone in the logarithmic-growth phase than the same strain in the stationary-growth phase.

An important factor in the interpretation of ozone inactivation curves is the experimental protocol used. In ozone demand-free work careful preparation of the test microorganism is required to prevent extraneous ozone demands and competing reactions which could mask actual inactivation kinetics. Other important factors include the analytical method used for dissolved ozone and the method in which ozone is introduced to the reactor. A two-stage or continuous inactivation curve is characteristic of independent trials conducted in reactors where ozone is not being continually replenished (Katzenelson *et al.*, 1974; Finch *et al.*, 1988; Wolfe *et al.* 1989a; Yuen, 1990). In their research, Wickramanayake *et al.* (1984a; 1984b) used a semi-batch reactor in which ozone was being continuously supplied to maintain a constant ozone residual. They reported that ozone inactivation of *Giardia* to the 99 percent inactivation level followed the Watson model; however, this may not have provided an accurate description of the kinetics because one of the reactants, ozone, was continuously replenished during the reaction. When ozone is continuously supplied, the ozone utilized for disinfection may be confounded with the ozone demand of the system, or extraneous demand associated with the test organism.

In general, investigators using bacteria and viruses as test microorganisms have found that ozone inactivation is relatively independent of contact time after an initial stage of about 1 min in clean water (Coin *et al.*, 1964; Farooq *et al.*, 1977; Finch *et al.*, 1988; Katzenelson *et al.*, 1974; Roy *et al.*, 1982). It can be concluded from these studies that ozone applied dose or residual concentration may be more important than contact time as a design factor.

2.4.5 Ozone Inactivation of *Giardia*

Molecular ozone is a very effective biocide, and has a specific coefficient of lethality towards amoebic cysts that is an order of magnitude higher than HOCl (Bablon *et al.*, 1991b). The most widely cited work on ozone inactivation of *Giardia* is that of Wickramanayake (1984) and Wickramanayake *et al.* (1984a; 1984b; 1985) who used *in vitro* excystation for viability determination and a semi-batch reactor (batch liquid, continuous gas) that maintained a constant ozone residual. They reported Ct_{99} values of 0.55 and 1.9 mg·min/L for *G. lamblia* and *G. muris*, respectively, at pH 7 and 5°C. The Ct_{99} values were 0.17 and 0.25 mg·min/L for the respective organisms at pH 7 and 25°C. *G. muris* was 3.5 times more resistant to ozone than *G. lamblia* at 5°C and 1.5 times more resistant at 25°C on the basis of Ct_{99} values. This marked temperature dependence of the sensitivity to ozone was not discussed by the authors. Others have suggested that there is little difference between *G. muris* and *G. lamblia* Ct_{99} values following ozonation at temperatures ranging from 6.7 to 9.0°C (Wallis *et al.*, 1990).

Wickramanayake *et al.* (1985) observed that *G. muris* cysts suspended in ozone demand-free water buffered at pH 7 was 7.6 times more resistant to ozone at 5°C than at 25°C. *G. muris* cysts were most sensitive to ozone at pH 9, then pH 5 and least sensitive at pH 7 (Wickramanayake *et al.*, 1984b). In the experimental design pH and temperature were varied one at a time, and information was not obtained on the temperature x pH interaction. The coefficient of dilution, n, was reported to be 1.1 and 1.2 for ozone inactivation of *G. muris* cysts (Wickramanayake *et al.* 1984b). This suggests that ozone concentration may be more important than contact time.

More literature on ozone inactivation of *Giardia* appeared in the late 1980s describing work performed at a large utility in California (Wolfe *et al.*, 1989a; Wolfe *et al.*, 1989b; Scott *et al.*, 1992; Ferguson *et al.*, 1990; Scott *et al.*, 1989; Metropolitan Water District of Southern California and James M. Montgomery Consulting Engineers, 1991). *In vitro* excystation was used for viability determination in a pilot-scale investigation of the disinfection efficacy of ozone–hydrogen peroxide and ozone in two untreated surface waters (pH 8; 14°C). They reported 99 percent *G. muris* inactivation with ozone residuals of 0.24–0.4 mg/L and contact times of 3–12 min. At an applied ozone dose of 1.0 mg/L, ozone and ozone–hydrogen peroxide required 8 and 9 min of contact time, respectively, to inactivate 99 percent of the *G. muris* cysts. Ct_{99} for *G. muris* was calculated as 3.4–5.4 mg·min/L using a variety of definitions for C and t. These values were 5.4–8.6 times greater than the USEPA values for *G. lamblia* at 15°C (Table 1.2). The Ct_{99} *G. muris* values were also 1.8–22 times greater than those reported by Wickramanayake *et al.* (1985), presumably due to differences in water quality and experimental protocol.

Different strains of *Giardia* appear to have different susceptibilities to chemical disinfectants. Rubin *et al.* (1989) used free chlorine and reported that gerbil-cultured *G. lamblia* cysts had Ct_{99} values of 120–1,495 mg·min/L. The Ct_{99} values were 27–50 mg·min/L for human-derived *G. lamblia* cysts inactivated under similar conditions in an earlier study (Jarroll, 1988). Human-derived *G. lamblia* cysts inactivated by ozone had Ct_{99} values of 0.17–0.55 mg·min/L (Wickramanayake *et al.*, 1984a).

2.5 ENGINEERING ASPECTS OF OZONE SYSTEMS

There are over 2,000 ozone water treatment installations worldwide, the majority being located in Europe (Tate, 1991). For a variety of reasons including better quality surface waters, lower expectations of consumers, and some design and performance problems, only approximately 90 of these are in North America. However, in the U.S. this number can be expected to increase as utilities prepare to meet the SWTR and the proposed D–DBP Rule. Most Canadian ozone plants are in Quebec where ozone is primarily applied after filtration for disinfection, following the French practice (Gomella, 1972). In Alberta, Canada ozone may be used as a pre-disinfectant or as an oxidant for removal of taste and odor. However, ozone may not be used as a disinfectant in the absence of chlorine under any circumstances (Alberta Environment, 1988). In the U.S. ozone is generally used as pre-treatment for raw water for D–DBP control or taste and odor control (Tate, 1991). A summary of the uses of ozone in water treatment is presented in Table 2.6. It is important to realize that although ozone may be installed for a single purpose, multiple benefits may accrue.

Increasingly important in the engineering design of ozonation for disinfection, and indeed, the use of other disinfectants alone or in combination with ozone, is the trade-off between microbial risk and D–DBP risk. More attention is being focused on the location of the disinfectant(s) in the overall treatment process. The area where microbial and D–DBP risks are most economically balanced is referred to as the regulatory range. At this time microbial risks are largely proven and serious, while many D–DBP risks are unknown. The by-products of chlorine, chlorine dioxide, chloramine, and ozone have been reviewed; however, much work remains to be done in identification, analytical methods, and determination of health risks (Rice and Gomez-Taylor, 1987). The by-products of ozonation are generally more biodegradable than their precursors (Rice *et al.*, 1981; Singer, 1990). Consequently there is a need for biological treatment to make the water biologically stable prior to distribution.

Classes of ozonation by-products include: aldehydes, epoxides, peroxides, nitrosamines, bromate and iodate (Glaze, 1986; Rice, 1991). Oxalic acid and acetic acid are the final oxidation products of ozone with many aliphatic and aromatic organic materials (Rice and Gomez-Taylor, 1987). Formic acid and formaldehyde are also formed; however, these are rapidly oxidized to carbon dioxide and water upon further ozonation (Rice and Gomez-Taylor, 1987). In the absence of free or combined chlorine, ozone does not form high levels of trihalomethanes unless

Table 2.6 Summary of ozone applications in water treatment (after Singer, 1990*)

Application	References
protozoan, viral and bacterial inactivation	Newton and Jones, 1949; Wickramanayake, 1984; Coin *et al.*, 1964; Sproul *et al.*, 1979; Roy *et al.*, 1982; Scott and Lesher, 1963; Katzenelson *et al.*, 1974; Jarroll, 1988*; Sobsey, 1989*.
oxidation of organic contaminants	Hoigné and Bader, 1975; Hoigné, 1982.
partial oxidation of organics for subsequent biological treatment	Rice *et al.*, 1981*; Singer, 1990*.
source of free radicals in advanced oxidation processes	Duguet *et al.*, 1989; Scott *et al.*, 1989; Ferguson *et al.*, 1990.
oxidation of soluble iron and manganese	Bablon *et al.*, 1991b*.
taste and odor control	Ferguson *et al.*, 1990; Glaze *et al.*, 1990; Bablon *et al.*, 1991b*.
coagulation aid	Bablon *et al.*, 1991b*.
algal lysis	Bablon *et al.*, 1991b*.

*review article

bromide is present. In this case bromoform is formed. Other ozonation by-products of concern include dibromoacetonitrile and dibromoacetic acid.

Bromate is the ozonation by-product of greatest concern because Japanese research has indicated that 5 μg/L levels of bromate ion can cause cancer in test animals (Bull and Kopfler, 1991). However, bromate chemistry is complex. Bromide first oxidizes to hypobromite ion, which rapidly hydrolyzes to hypobromous acid (Rice and Gomez-Taylor, 1987). Only hypobromite is further oxidized to bromate.

2.5.1 Terminology

The nature of ozone contacting and mass transfer has led to the use of various metameters for ozone concentration (IOA, 1990; Bablon *et al.*, 1991a):

1. Applied ozone is the mass of ozone applied to the reactor.
2. Transferred ozone is the mass of ozone transferred from the gas phase to the liquid phase divided by the treatment volume. This definition of transferred ozone is not appropriate if dissolved ozone is being added to batch reactors rather than ozone gas in full-scale or pilot-scale reactors. When ozone is added to batch reactors in solution rather than as a gas, transferred ozone may be taken as equal to the applied dose.
3. Residual ozone is ozone concentration at the end of the contact period.
4. Utilized ozone dose is defined as transferred ozone dose less residual ozone.

The USEPA has published a "Guidance Manual for Compliance with the Filtration and Disinfection Requirements for Public Water Systems Using Surface Water Sources" (USEPA, 1991). Appendix G.4 of the *Guidance Manual* provides information on determining the ozone inactivation of *Giardia* cysts and viruses using either a pilot study or a batch system. Appendix O provides recommendations on the definitions of C and t in ozone disinfection. A theoretical evaluation of the characteristic C and t definitions in ozone disinfection is available elsewhere (Lev and Regli, 1992a; Lev and Regli, 1992b). A review of the *Guidance Manual* Appendix O recommendations follows.

2.5.2 Contact Time

Contactors designed for ozone disinfection have contact times that are typically 10 min or less (Joost *et al.*, 1989). The well-known French disinfection standard requires a 0.4 mg/L ozone residual at the end of a 4 min contact time (Coin *et al.*, 1964). It was found that this condition resulted in greater than 99.99 percent inactivation of poliovirus type 1 in filtered river water.

Using the hydraulic detention time (HDT) as t is only appropriate where the ozone contacting system operates under plug-flow conditions. However, a series of individual complete-mix chambers tends to approach plug-flow conditions (Levenspiel, 1972). The *Guidance Manual* recommends the use of t_{10} for the characteristic time t (USEPA, 1991). t_{10} is defined as the time that at least 90 percent of the liquid remains in the chamber. Typically this is determined from dye tracer tests for the time of 10 percent breakthrough relative to the overall contactor HDT (Rakness *et al.*, 1991). The contactor is operated with oxygen or air during the tracer test instead of ozone (USEPA, 1991).

Because bubble diffusers and aspirating turbines have very different hydraulic characteristics and are both commonly used ozone contactors for water treatment, the *Guidance Manual* provides a number of options for determining t (Rice, 1991; USEPA, 1991):

1. The t_{10} method. This is the preferred method, except when more than 2.5 log ozone inactivation of *Giardia* cysts is required, or the ratio t_{10} to HDT is less than 0.33. The main drawback of the t_{10} method is that, unlike HDT, it is nonlinear (Lev and Regli, 1992b).

2. Segregated Flow Analysis. This method is often used to characterize chemical reactions. A high resolution tracer study is required to define the probability distribution of water remaining in the contactor. Since each fraction of flow has a different "t", a modified form of Chick's equation is used to define a second probability distribution for organism survival through the reactor. The product of these two probability distributions is then used to determine microorganism inactivation.

3. Continuously Stirred-Tank Reactor (CSTR). This is the most conservative approach. It should only be used if high microorganism inactivation is required in a single chamber contactor and flow data is not available. The method assumes that the microorganisms and disinfectant are homogeneously distributed in the contactor. The first-order Chick-Watson model is applied directly rather than using the Ct approach. The *Guidance Manual* provides equations for determining CSTR performance, however the effects of mixing, which can be a significant factor in disinfection performance in CSTR reactors, are not included in the equations.

Two engineering studies of multi-chamber bubble diffuser ozone contactors have shown that t_{10} was 50 and 50.9 percent of HDT for six chambers in series (Joost *et al.*, 1989; Rakness *et al.*, 1991). The theoretical value for six identical chambers in series is 52 percent (Roustan *et al.*, 1991). In a step-input and pulse-input tracer study of chlorination performance at three water treatment plants, Teefy and Singer (1990) reported that the t_{10} to HDT ratio varied from 0.15 to 0.52. The higher ratios were for reservoirs that contained more tanks in series, or interior baffles, and therefore exhibited less short-circuiting. Effective Ct values were calculated based on residence time distribution and the kinetics of chlorine decomposition and were found to be up to six times the Ct_{10} values provided for by the SWTR (Teefy and Singer, 1990). The t_{10} to HDT ratio is 0.1 for a CSTR and 1 for a plug-flow reactor, the two ideal cases (Roustan *et al.*, 1991). Current engineering design of ozone contactors for primary disinfection in water treatment favours multi-chamber bubble diffuser units since they increase plug-flow conditions minimizing contactor volumes as well as short-circuiting (IOA, 1990).

It is important to note the method in which t is calculated when comparing ozone inactivation Ct values reported in the literature. In a recent study t_{50}, or the time to reach 50 percent breakthrough was used in addition to t_{10} (Wolfe *et al.*, 1989b). In most cases t_{50} is an inferior measure of t since it does not guarantee SWTR compliance; however, in perfect plug-flow reactors $t_{10} = t_{50} =$ HDT (Lev and Regli, 1992b). Should the *Giardia* regulatory requirement be increased to 4 logs inactivation, t_{10} will not guarantee compliance in reactors with high amounts of backmixing (Lev and Regli, 1992b).

2.5.3 Ozone Concentration

Due to the instability of dissolved ozone in water, C values must be determined within the ozone contacting system itself. The USEPA classifies ozone contacting systems into four types depending on their flow regime (USEPA, 1991):

1. Continuously stirred-tank reactor (turbine agitators)
2. Cocurrent flow chambers (bubble diffusers)
3. Countercurrent flow chambers (bubble diffusers)
4. Reactive flow chambers (no ozone introduced, but ozone residual in water).

The use of an average concentration of ozone for all types of contactors, determined either by direct measurement or by indirect prediction as summarized in Table 2.7, is recommended. The following guidelines should be used in the direct measurement of C (USEPA, 1991):

1. analyze each chamber separately;
2. select representative locations;
3. avoid interference from gas bubbles;

Table 2.7 Methods for predicting C based on outlet ozone concentration[1,3] (USEPA, 1991; Rice, 1991)

	Flow Regime			
	Turbine	Cocurrent	Counter-current	Reactive
First chamber	C	partial credit[2]	partial credit[2]	not applicable
Subsequent chamber	$C = C_{out}$	$C = C_{out}$ or $C = (C_{out} + C_{in})/2$	$C = C_{out}/2$	$C = C_{out}$

[1] Definitions:

C is the characteristic concentration (mg/L)

C_{out} is the dissolved ozone concentration at the outlet from the chamber (mg/L)

C_{in} is the dissolved ozone concentration at the inlet to the chamber (mg/L)

[2] 0.5 log *Giardia* cyst inactivation provided that $C_{out} > 0.3$ mg/L

[3] Alternatively, C may be taken as the average concentration as evaluated by direct measurements

4. provide proper spatial distribution, with at least five vertical locations and at least two horizontal locations for each vertical sampling point within the contact chamber; and
5. minimize distance to ozone analyzers.

In many ozone contactors, it is not practical to measure ozone profiles directly. The characteristic C may then be estimated from residual ozone measurements at chamber outlets as shown in Table 2.7. Generally, excepting the first chamber and countercurrent flow, C in multi-chamber contactors is the exiting ozone residual for the chamber in question.

In the countercurrent flow configuration, for waters having little ozone demand and linear kinetics, the ratio of the average ozone concentration to the exiting ozone concentration lies between 1/3 and 1/2 (Lev and Regli, 1992a). The ratio is about 0.33 when mass transfer is dominant and 0.40 when ozone decomposition is dominant, assuming ozone transfer efficiencies of approximately 90 percent (Lev and Regli, 1992a). The "one-third rule" does not apply for nonlinear kinetics (the dominant kinetic mechanism may become second-order in a water with high ozone demand), nonzero inlet ozone concentration, or high turbulence (Lev and Regli, 1992a).

To prevent costly overdesign and possible ozone overdosing, the USEPA permits site-specific evaluations and recommends the following alternatives which require better characterization of ozone contactor hydrodynamics (USEPA, 1991):

1. Estimating C by measurement of another observable parameter such as off-gas ozone or C_{out} and developing a specific correlation. Providing that the transfer efficiency is greater than 85 percent, the USEPA allows water systems to use solubility constant data to calculate C_{out} based on the ozone concentration in the off-gas. Interpolation is permitted with the developed correlation, but not extrapolation.

2. Using mathematical models of field-scale ozone contactors to predict disinfection performance is an alternative to the separate C and t approach. The model must specific to individual chambers and be verified by experimental measurements of the actual ozone profile in the contact chambers. The model should account for the ozone demand of the water being tested.

3. Use of microbial indicator studies, for example, *G. muris* for *G. lamblia*. Methods are presented to directly or indirectly determine inactivation performance assuming inactivation kinetics approximate Chick's law. Batch experiments are encouraged.

Roustan *et al.* (1991) compared the Ct approach with a multiple CSTR in series model which considered the kinetics and hydraulics of the ozonation process. They concluded that to obtain 99 percent inactivation of *Giardia* cysts, a reactor with a hydraulic behavior of at least 3 CSTRs in series was necessary. To obtain 99.9 percent inactivation, a reactor with a hydraulic behavior of at least 5 CSTRs in series was necessary; meaning a reactor with two compartments, each having the behavior of 2 to 3 CSTRs in series (Roustan *et al.*, 1991).

$C = C_{out}$ or residual ozone has been most frequently reported in the literature; however, a countercurrent contactor pilot-scale study reported while the *Guidance Manual* was still in draft form used both residual ozone and average ozone concentration [(ozone residual + ozone dose)/2] for C (Wolfe *et al.*, 1989b). The average ozone definition resulted in higher calculated Ct values; however, the real contribution of ozone dose to the characteristic C is highly dependent on ozone demand and reaction rate coefficients (Lev and Regli, 1992a).

3. RESEARCH OBJECTIVES

As a result of the literature review, the primary research objectives of this investigation were:

1. to determine if direct or hydroxyl radical-mediated reactions are more important in the ozone inactivation of *Giardia;*
2. to determine the importance of selected process and water quality variables on the ozone inactivation of *Giardia* in laboratory waters;
3. to perform bench-scale and pilot-scale ozonation experiments using selected natural surface waters with *Giardia* cysts added; and
4. to develop a protocol enabling a utility to calculate the Ct, or equivalent, for ozone applied prior to filtration.

Secondary research objectives were:

1. to compare *in vitro* excystation, fluorogenic dye staining, and animal infectivity as measures of *Giardia* cyst viability following ozonation in laboratory waters; and
2. to compare the inactivation of *G. muris* and *G. lamblia.*

4. MATERIALS AND METHODS

4.1 EXPERIMENTAL WATERS

Two classes of water were used. The first class of water was carefully controlled laboratory waters which were used as a bench-mark for comparing results with previous literature and as a quality control measure for work on this project. The laboratory waters used were deionized water, 0.05 M phosphate buffer and 0.05 M phosphate-0.01 M bicarbonate buffer. The second class of water was natural surface waters of good quality.

4.1.1 Laboratory Waters

High quality deionized water was obtained from a Milli-Q® system (OM-140; Millipore Corp.) operated at a resistivity of at least 18 MΩ/cm. Deionized water from this apparatus typically had a pH of 6.9. Preliminary experiments (exps. 13 to 28) were conducted using ozone demand-free, deionized water due to the relatively stable ozone residual which can be maintained when initiators or promoters of ozone decomposition are not present in significant concentrations. Deionized water was also used for preparing all analytical and test solutions including the stock ozone solution.

A 0.05 M phosphate buffer was used for suspending cysts in all work subsequent to experiment 28 except for the trials in experiment 31 where 0.05 M phosphate-0.01 M bicarbonate buffer was used. Phosphate buffer was prepared using potassium dihydrogen orthophosphate and disodium hydrogen orthophosphate to provide the required pH (AnalaR grade; BDH). Lower grades of reagent adversely affected the half-life of ozone in solution. Typically 50.0 mL of a 1.0 M stock solution and 950.0 mL of Milli-Q® water were added to a 1,000.0 mL volumetric flask to provide a 0.05 M solution. The 0.05 M phosphate-0.01 M bicarbonate buffer was prepared by a 1 in 20 dilution of a stock solution consisting of 127.92 g KH_2PO_4, 8.43 g Na_2HPO_4 and 16.80 g $NaHCO_3$ (AnalaR grade;

BDH) dissolved in 1,000 mL of Milli-Q® water. The diluted phosphate-bicarbonate buffer was pH 6.4.

4.1.2 Natural Waters

Natural surface waters were obtained from the North Saskatchewan River (NSR) at the raw water intake of the City of Edmonton's E.L. Smith Water Treatment Plant and the Greater Vancouver Regional District (GVRD) from the Capilano Reservoir. The characteristics of these waters are summarized in Table 4.1. The pH, turbidity, total alkalinity, total hardness and total organic carbon were measured by methods 4500-H^+B, 2130B, 2320B, 2340C, and 5310C, respectively (APHA *et al.*, 1989). Dissolved organic carbon was determined by filtering samples through a 0.45 μm glass fiber filter with vacuum prior to analysis. Absorbance at 260 nm was determined using a 1 cm light path length cuvette and a diode-array spectrophotometer (8452A; Hewlett-Packard). Conductivity was determined with a YSI Model 33 conductivity meter at 20°C. Heterotrophic plate count (20°C, day 7) and total coliform bacteria were determined by method 9215D using R2A agar and method 9222B, respectively (APHA *et al*, 1989). An approximation of ozone demand at 5°C was calculated by comparing the ozone demand characteristics of natural water with that of the demand-free buffer reference. Immediate ozone demand and a portion of 5 min decomposition were summed. An example of this calculation follows using the absorbance versus time data in Figure 4.1. The peak ozone concentration can be calculated as (0.300 – 0.059) x 14.55 = 3.51 mg/L, where 14.55 is the concentration factor when 3,300 M^{-1} cm^{-1} is used as the molar absorption coefficient. Applied ozone was 4.35 mg/L, calculated from the stock ozone solution concentration and the mass calibrated volume of stock added to the reactor. The resulting peak-to-applied ratio was 0.81. In ozone demand-free trials performed both with and without cysts, the mean value for the peak-to-applied ratio was found to be 0.90. Thus the immediate ozone demand is calculated as (0.90 – 0.81) x 4.35 = 0.39 mg/L. In Figure 4.1, it was hypothesized that the nonlinear portion of decomposition was also due to ozone demand, because in many of the demand-free trials decomposition in this plot

Table 4.1 Water quality characteristics of North Saskatchewan River (E.L. Smith Water Treatment Plant, Edmonton, Alberta) and Capilano Reservoir (Greater Vancouver Regional District, Vancouver, British Columbia) waters

Parameter	North Saskatchewan River				Capilano Reservoir	
Date of Sample	14-11-90	14-01-91	31-01-91	13-02-91	22-02-91	13-03-91[#]
TOC (mg/L)	7.04	1.23	1.62	1.79	11.12	ND
DOC (mg/L)	ND	0.99	ND	1.61	9.15	ND
Absorbance (260 nm, cm^{-1})	ND	0.070	0.068	0.056	0.113	0.102
Conductivity (µmhos/cm)	325	310	280	300	10	ND
pH	8.23	8.34	8.30	8.08	6.31	6.32
Total Alkalinity (mg as $CaCO_3$/L)	130	152	142	125	0.91	ND
Total Hardness (mg as $CaCO_3$/L)	176	179	176	160	6.05	ND
Heterotropic Plate Count[*] (CFU/mL)	ND	5,800	5,100	10,450	ND	ND
Total Coliforms (CFU/100 mL)	130	90	61	303	ND	ND
Turbidity (NTU)	6	2	3	2.6	3.8	3.9
Ozone Demand[†] (mg/L)	ND	0.94	ND	ND	0.81	0.49

[*] 20°C, day 7, R2A agar;
[†] 5°C;
[#] Reanalysis of 22-02-91 sample for selected parameters;
ND - Not determined.

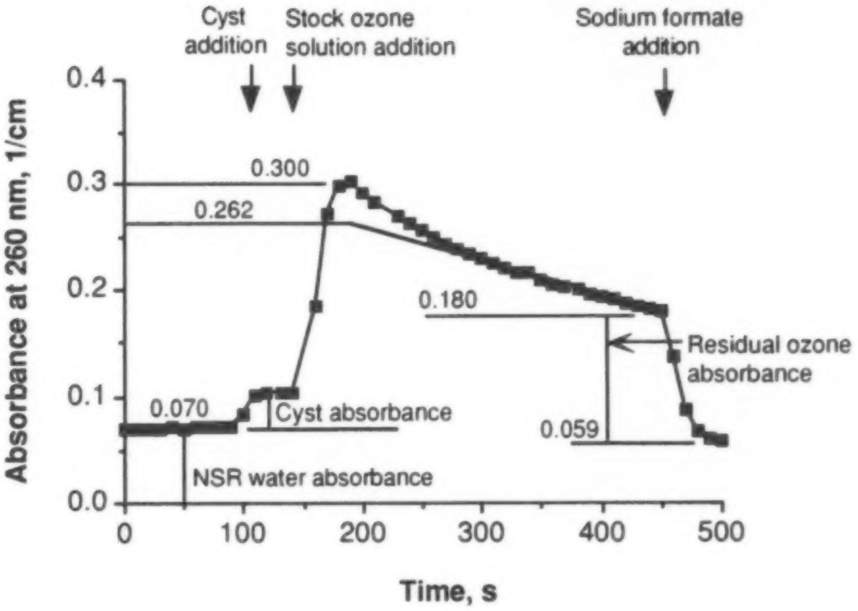


Figure 4.1 Ozone decomposition in North Saskatchewan River (NSR) water (5°C, with *G. muris* cysts)

was linear. This portion of the demand is calculated as (0.300 - 0.262) x 14.55 = 0.55 mg/L, and the total ozone demand is 0.94 mg/L, the value shown in Table 4.1 for the 14-01-91 sample. Since this work was done, a proposed standard method has been published for ozone demand which is somewhat different than that used here (APHA *et al.*, 1991).

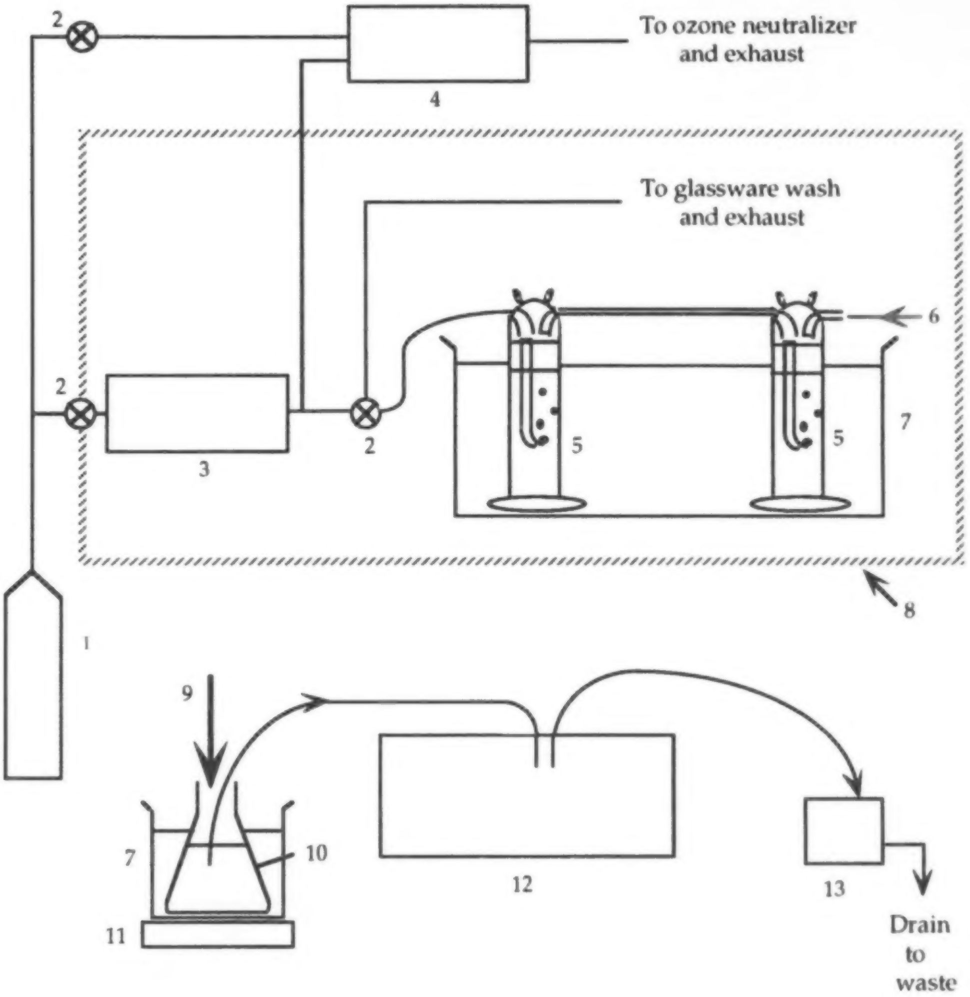
4.2 OZONE APPARATUS AND METHODS

4.2.1 Generation and Residual Determination

4.2.1.1 Bench-scale experiments

Ozone was generated from extra-dry oxygen using a corona discharge generator (C2P-9C; PCI Ozone Corp.). Ozone concentration in the carrier gas was approximately 4.8 percent ozone (wt/wt) at ambient temperature and pressure and was determined by u.v. spectrophotometry (HC12; PCI Ozone Corp.). The ozone-oxygen gas mixture was bubbled for a minimum of 20 min through 400 mL of 22°C Milli-Q® water in a 500 mL gas absorption flask. The stock ozone solution concentration was typically 18 to 20 mg/L in laboratory water experiments. In natural water experiments the gas absorption flask was immersed in an ice-water bath (4°C) and up to 42 mg/L was obtained in the stock ozone solution. Figure 4.2 illustrates the ozone generation and residual determination apparatus.

The concentration of ozone in aqueous solution was continuously determined using a diode-array spectrophotometer (8452A; Hewlett-Packard) at 260 nm and using a molar absorption coefficient of 3,300 M^{-1} cm^{-1} (Gordon *et al.*, 1988). Sample absorbance was integrated for 5 s with a 10 s cycle time. Residual ozone was neutralized in the reactor at the end of the contact period using 50 µL of 1.0 M sodium formate (Figure 4.1). Sodium formate did not have any interfering absorbance at 260 nm as was the case with sodium thiosulphate nor did it reduce the absorbance of the cyst-natural water mixture when added to natural water control trials. Preliminary work (exps. 13 to 28) used air-purging to


2
To ozone neutralizer and exhaust
4
To glassware wash and exhaust
6
2
3
2
5
5
7
8
1
9
7
10
11
12
13
Drain to waste

1. Extra-dry oxygen
2. Needle valve
3. Ozone generator
4. Ultraviolet monitor
5. Gas absorption flask
6. Back pressure control
7. Ice-water bath
8. Fume hood
9. Add stock ozone solution
10. Batch reactor (250mL)
11. Magnetic stirrer
12. Diode-array spectrophotometer
13. Pump

Figure 4.2 Ozone generation, residual determination and reaction apparatus (reactor ice-water bath used for 4°C experiments; generation ice-water bath used in natural water experiments).

neutralize ozone residual and ozone concentration was determined using u.v. spectrophotometry (Spectronic 601; Milton-Roy).

The concentration of hydrogen peroxide in aqueous solution was determined using the cobalt method (Masschelein *et al.*, 1977). The hydrogen peroxide residual calibration curve is provided in Appendix A. Residual hydrogen peroxide was neutralized using 0.600 mL of 20,000 enzyme units/mL catalase (from bovine liver in a solution containing glycerol and ethanol; BDH).

4.2.1.2 Pilot-scale experiments

Ozone was generated from extra-dry oxygen by a corona discharge ozone generator (T-816; Welsbach). Ozone concentration in the carrier gas was monitored for a 10 min period to check ozone generator stability prior to trials and was typically (mean ± SD) 2.65 ± 0.03 percent ozone (wt/wt). During trials ozone concentration in the carrier gas and off-gas were continuously determined by u.v. spectrophotometry and were varied according to the ozone demand of the water and the desired ozone dose. This permitted the ozone mass involved in the reaction to be very accurately calculated (Finch and Smith, 1989). Ozone residual was determined using the indigo trisulfonate method (APHA *et al.*, 1989). Residual ozone in 1 L aliquots taken from the pilot-scale reactor was neutralized using an excess of sodium formate.

4.2.2 Ozone Reactors

4.2.2.1 Bench-scale experiments

Three types of bench-scale batch reactor vessels were used:

1. A 50 mL reactor which was a 50 mL Erlenmeyer flask containing 41 mL of liquid.
2. A 250 mL reactor which was a 250 mL Erlenmeyer flask containing approximately 200 mL of liquid.
3. A 2 L borosilicate glass beaker with a stainless steel quadrant baffle and floating Teflon® cover containing 1.6 L of liquid.

The 250 mL Erlenmeyer flask batch reaction vessel was used exclusively for all work except preliminary experiments. The 250 mL reactor provided an optimal combination of number of cysts required for a single trial, cyst recovery, centrifugation effort, and stable ozone residual. Reaction vessels were covered with aluminium foil during ozonation experiments to prevent interference in ozone decomposition from stray u.v. radiation. Mixing was accomplished using a Teflon®-coated magnetic stir bar. In laboratory water experiments reactors were made ozone demand-free prior to use as follows:

1. Glassware, magnetic stir bars, tongs, etc. were acid-washed (6 mL of 25 percent phosphoric acid) using an automatic lab dishwasher (G7733; Mielabor) set for one wash cycle and two 85°C distilled water rinse cycles. Soap was not used.
2. Glassware was rinsed with Milli-Q® water, dried, then covered with aluminium foil to prevent dust accumulation.
3. A magnetic stir bar and medium was added to reaction vessel, which was then ozonated at high capacity (C2P-9C generator; PCI Ozone Corp.) for at least 5 min/L of solution using a glass esparger. Mixing was done at the same time.
4. Reactor was left to sit in a fume hood for at least 1 h.
5. Excess ozone was driven off by autoclaving or letting the vessel stand overnight and confirming that all ozone has dissipated before use.

Just steps 1 and 2 above were required in natural water experiments.

4.2.2.2 Pilot-scale experiments

The pilot-scale reactor was a 100 L semi-batch (continuous gas, batch liquid), stirred tank reactor that provided a 400-fold scale-up of the bench-scale reactor. It was constructed from type 316 stainless steel and sealed using Teflon® gaskets. Hydraulic conditions in this reactor approached plug-flow conditions. Ozone entered through the bottom of the vessel as a gas and was dispersed throughout the vessel by a sparger and rotating paddle. The bubble size in the reactor was adjustable by changing the power input to the contactor by means of the variable speed motor and Rushton turbine as described previously (Finch and Smith, 1989). There are few sources of cyst loss in this reactor because it is a batch reactor where the liquid is homogeneously mixed. A valved port was used to sample liquid from the middle of the vessel. Mass-transfer and mixing are two of the most important variables in ozone disinfection, and the semi-batch, stirred tank reactor was well suited for conclusions regarding the scale-up of these factors.

4.3 *GIARDIA* METHODS

4.3.1 Parasites

4.3.1.1 *Giardia muris*

The strain of *G. muris* used in this study was originally isolated by Roberts-Thomson (Roberts-Thomson *et al.*, 1976a; 1976b) from a golden hamster and was obtained from B.J. Underdown, McMaster University, Hamilton, Ontario, Canada. The parasite has been maintained in the laboratory since 1981 using bi-weekly passages in CD-1 or C3H/HeN mice. The *G. muris* culture is free of all other contaminating intestinal protozoa including *Hexamita* as shown by fecal and small intestine examinations using light microscopy and histology.

4.3.1.2 *Giardia lamblia*

Trophozoites of WB isolate of *G. lamblia* (ATCC 30957, isolated from a duodenal aspirate of a human male) were used for initial infection. This isolate is routinely maintained *in vitro* using Diamond's TYI-S-33 medium (Belosevic *et al.*, 1982). Actively growing *G. lamblia* trophozoites (48- to 96-h-old culture) were incubated on ice for 15 min to detach the parasite from the walls of the screw-capped 16 x 125 mm culture tube. The organisms were then sedimented by centrifugation at 200 x *g* for 10 min, washed in phosphate-buffered saline (PBS), and resuspended in 0.2 mL of PBS to contain 1×10^5 trophozoites. Gerbils were inoculated orally with 0.2 mL of PBS containing 1×10^5 *G. lamblia* trophozoites. Subsequently, *G. lamblia* was maintained by cyst passage through gerbils (Belosevic *et al.*, 1983).

4.3.2 Animals

4.3.2.1 Mice

Specific-pathogen-free (SPF) 6- to 8-week-old, male, inbred C3H/HeN mice were used and were obtained from Charles River Breeding Laboratories (St. Constant, Que.) until November 1990. Beginning January 1991, the animals were obtained from Taconic Laboratories Inc. (German Town, N.J.).

Upon arrival, mice were kept under SPF conditions (laminar flow isolator) and were given sterile food and water *ad libitum*, housed 4 to 5 per cage, had sterile bedding changed twice per week and were maintained with a diurnal cycle of 14 h of light and 10 h of dark. The access to the laminar flow isolator was limited to two animal-care technicians. Animals to date have been found to be free of natural *Giardia* infections as shown by fecal examinations for cysts and small intestine examination for trophozoites of randomly chosen animals. Following infection with *G. muris* the mice were individually housed in filter-top (0.22µm filter) cages in a separate SPF isolation room.

4.3.2.2 Gerbils

Six- to 10-week-old male Mongolian gerbils (*Meriones unguiculatus*) were used in all experiments. These animals were purchased from Tumblebrook Farms (West Brookfield, Mass.). Upon arrival at the animal facility, gerbils were placed in filter-top cages (one gerbil per cage) and treated for 3 consecutive days with 20 mg/day/gerbil of Metronidazole (Flagyl®; Rhone-Poulenc, Montreal, Que.), administered by gavage. This treatment ensured that gerbils were free from all previous intestinal infections (Belosevic *et al.*, 1983; Faubert and Belosevic, 1990).

4.3.3 Isolation of Cysts

4.3.3.1 *Giardia muris*

G. muris cysts were isolated from the feces using the modified sucrose gradient centrifugation technique (Roberts-Thomson *et al.*, 1976a; Belosevic and Faubert, 1983a). Feces were collected 7 days after infection from 10 to 15 mice over a period of 2 to 3 h. Feces were soaked for 30 min in Milli-Q® water, emulsified by using applicator sticks, layered on a sucrose solution (spec. grav. 1.12), and centrifuged for 15 min at 400 x *g* in a swinging-bucket centrifuge (GR4.11; Jouan) at 4°C. After centrifugation, cysts trapped at the water-sucrose interface were carefully removed with a pipette and washed in Milli-Q® water by centrifugation at 600 x *g* for 10 min. The pellet containing the cysts was resuspended in a known volume of Milli-Q® water and the number of cysts in the sample determined by counting 4 complete grids of a hemocytometer.

In order to obtain highly purified cysts suitable for use in ozone demand-free experiments, cysts were isolated as above; however, 2 to 4 additional Milli-Q® water washes were required. The absorbance of a cyst suspension of 10^4 cysts/mL at 260 nm was used as a quality control measure. Only cysts with an absorbance less than 0.03/cm were used in experiments (Figure 4.3). The final cyst preparation was resuspended to the desired concentration using ozone demand-free Milli-Q® water. The number of cysts in the sample was determined by counting 4 complete grids of a hemocytometer. Cysts were stored at 4°C for 18 h and were used within 48 h of preparation. It was found that storage of cysts at 4°C for longer than 48 h resulted in decreased viability of the organisms.

4.3.3.2 *Giardia lamblia*

Cysts released in a 4 h fecal collection (9:00 a.m. to 1:00 p.m.) from 15 gerbils were collected in 12 x 75 mm glass tubes from day 7 to day 14 after infection. Cysts were separated from the feces by filtration through 180 and 45 μm stainless-steel sieves and concentrated using the sucrose gradient centrifugation technique described above. Cysts were washed

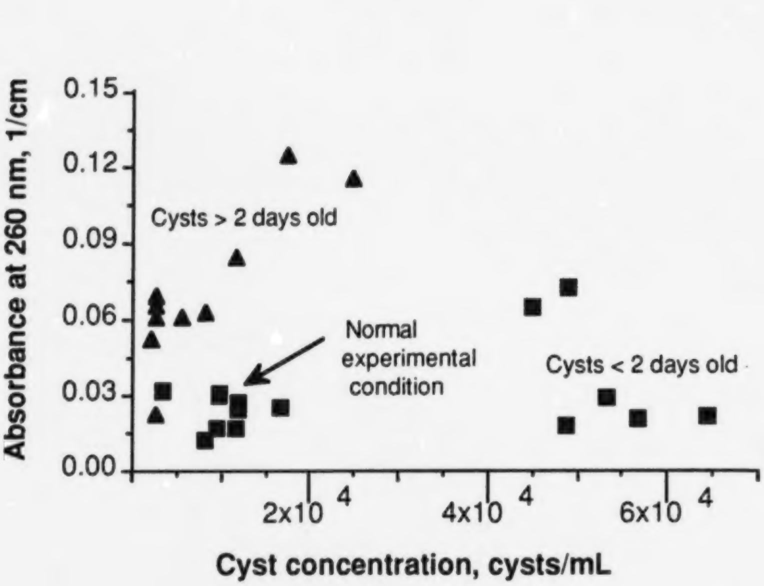


Figure 4.3 Effect of cyst age on absorbance of cyst preparation

three times in Milli-Q® water by centrifugation at 200 x *g* for 10 min (GR4.11; Jouan) so that the absorbance at 260 nm of a cyst suspension of 10^4 cysts/mL did not exceed 0.03/cm. The final cyst preparation was resuspended to 10^6 cysts/mL using ozone demand-free Milli-Q® water. The number of cysts in the sample was determined by counting four complete grids of a hemocytometer.

4.3.4 Isolation of Trophozoites

The upper 25 percent of the small intestine was placed in 3 mL of PBS (Gibco). Four to 10 mucosal scrapings from this segment were placed on glass microscope slides and examined for the presence of trophozoites by using a bright-field microscope.

4.3.5 Fluorogenic Staining

A modified FDA-EB staining technique was used (Jackson *et al.*, 1985). FDA stock solution (5 mg/mL in acetone; ICN Biochemicals Corp.) and EB stock solution (20 µg/mL) in phosphate-buffered saline (Sigma Chemical Corp.) were stored at -30°C prior to use. A working dilution of FDA was made by adding 20 µL of stock FDA to 10 mL of Milli-Q® water. A 100 µL aliquot of *G. muris* cysts was added to a 1:1 solution of diluted FDA (50 µL) and stock EB (50 µL). The mixture was incubated at room temperature for 5 min, and the reaction was then stopped by adding 200 µL of Milli-Q® water. Both sides of the hemocytometer were loaded with a well-mixed stained cyst suspension and the cysts were allowed to settle to the bottom of the hemocytometer chamber for 60 s. The cysts were counted first under incident light and then under u.v. light by using an epifluorescence microscope (Optiphot; Nikon) equipped with an EX330 excitation filter and a BA435 barrier filter. A minimum of four complete grids of the hemocytometer were counted for each sample, and counting was completed within 1 h.

4.3.6 Excystation

A procedure described by Schaefer *et al.* (1984) was used to determine excystation. Between 1×10^5 and 5×10^5 purified *G. muris* cysts contained in 0.5 mL were placed into a 15 mL plastic, screw-cap, graduated, conical centrifuge tube. First, 5 mL of prewarmed (37°C) reducing solution was added, and then 5 mL of prewarmed (37°C) freshly prepared 0.1 M $NaHCO_3$ was added. The tube was vortexed vigorously, incubated in a 37°C water bath for 30 min, and centrifuged for 2 min at 400 x *g* in a swinging-bucket centrifuge, and the supernatant was aspirated down to 0.2 mL. The pellet was resuspended in 10 mL of chilled (4°C) trypsin-Tyrode's solution and vortexed vigorously. The tube was centrifuged for 2 min at 400 x *g* and the supernatant was aspirated down to 0.2 mL. Three-tenths of a mL of prewarmed (37°C) trypsin-Tyrode's solution was added to the tube, and the pellet was resuspended. Three to four drops of the suspension were placed in a chamber slide, the coverslip was sealed with a Vaseline®-paraffin mixture, and the slide incubated in a 37°C air incubator for 30 min. It was then examined with a phase-contrast microscope and a 40X phase objective lens. Empty cyst walls (ECW) and partially excysted trophozoites (PET) were counted as positive excystation; intact cysts (IC) showed no signs of excystation. The total number of ECW, PET, and IC examined was 1,000 or the number present in the entire slide area if it was not possible to find 1,000. The excystation procedure was completed within 3 h.

4.3.7 Animal Models

4.3.7.1 C3H/HeN mouse-*G. muris* model

The C3H/HeN mouse-*G. muris* model (Roberts-Thomson *et al.*, 1976a; Belosevic and Faubert, 1983a; Belosevic *et al.*, 1984) was used to assess *G. muris* cyst infectivity. Data on the effects of different doses of untreated cysts on the duration of the latent period in SPF male C3H/HeN mice are presented in Table 4.2. The data show a significant correlation between the size of the inoculum (cysts/mouse) and the time (days) when all mice in a given dose group were passing cysts. The discriminating variable is not the beginning of the latent period but rather the time when all mice in a group were releasing cysts in the feces. There were over 10 replications of the 10^5 and 10^4 cyst doses, and 2 replications for each of the 10^3, 10^2 and 10 cyst doses; all with identical

Table 4.2 C3H/HeN mice infected by *G. muris* as a function of days after infection and inoculum size

Inoculum Size (cysts per mouse)	No. of Mice in Group	Days After Infection* (No. of mice positive for cysts)									
		1	2	3	4	5	6	7	8	9	10
10^5	9	0	0	9	9	9	9	-	-	-	-
10^4	10	0	0	0	10	10	10	10	-	-	-
10^3	10	0	0	0	3	10	10	10	10	-	-
10^2	9	0	0	0	0	5	9	9	9	9	-
10	9	0	0	0	0	3	6	6	9	9	9
1	12	0	0	0	0	0	0	2	3	5	5†

*Data collected until day 10 or for 3 days after all mice in group became positive.

†Trophozoites detected in 9 of the 12 mice in this group.

results. Only 5 of 12 mice inoculated with 1 cyst were releasing cysts on day 10 after inoculation. However, the examination of the small intestine of these mice showed that 9 out of 12 mice (75 percent) were infected with trophozoites of *G. muris*, indicating that C3H/HeN mice are highly susceptible to infection.

4.3.7.2 Mongolian gerbil-*G. lamblia* model

The Mongolian gerbil-*G. lamblia* model was proposed by Belosevic *et al.* (1983). Characteristics of the model include: a susceptibility to infection with *G. lamblia* cysts that is much higher than that for either rats or dogs; a pattern of cyst release and duration of infection similar to that for the human infection; and the nontransient nature of the infection (Belosevic *et al.*, 1983). Cyst release is intermittent, with cysts first detected on day 8 for Mongolian gerbils infected with *G. lamblia* cysts, day 9 for gerbils orally inoculated with *in vitro* trophozoites, and day 12 for gerbils duodenally inoculated with *in vitro* trophozoites (Belosevic *et al.*, 1983). The latent period varied from 4 to 8 days depending on the inoculum size, and the duration of infection was 13 to 86 days; however, most cysts were observed during the second and third weeks. Maximal cyst output occured when the number of trophozoites present in the small intestine was highest (Belosevic *et al.*, 1983). It was concluded that the intermittent nature of cyst release is a feature of the parasite, rather than a host characteristic (Belosevic *et al.*, 1983; Faubert *et al.*, 1983). Mongolian gerbils acquire complete resistance when challenged by the same species of *Giardia*, but only partial resistance when challenged with a different species of *Giardia* and can therefore be used for the identification of the etiological agent as well as determining infectivity (Faubert *et al.*, 1983).

The intermittent nature of cyst release prevents a latent period approach to the determination of inactivation. A most probable number approach has been used (Haas and Heller, 1990; Hibler *et al.*, 1987); however, was beyond the scope of this study due to budget and time constraints. Rather a semiquantitative determination of infection was made using ID_{50} data from the literature (Schaefer *et al.*, 1991).

4.3.8 Infectivity Testing Procedures

Infectivity testing procedures adhered to the Medical Research Council of Canada and Natural Sciences and Engineering Research Council of Canada guidelines for animal care.

4.3.8.1 *Giardia muris*

All disinfected liquid was centrifuged in 175 mL plastic conical centrifuge tubes at 800 x *g* for 10 min. The supernatant was removed, leaving a 1.5 mL centrifugate, of which 1.0 mL was used for infecting mice. In the viability comparison experiment, the centrifugate was 2.1 mL, of which 1.0 mL was used for infecting mice, 0.5 mL was used for excystation, and 0.100 mL was used for FDA-EB fluorogenic staining; the remaining portion being surplus. A minimum of four hemocytometer counts were made on the 1.0 mL portion of the split sample in order to determine the total number of cysts. Each one of a group of 5 C3H/HeN mice was orally inoculated with 0.200 mL. The size of the inoculum was typically 10^4 cysts/mouse in the screening and ozone/ozone–hydrogen peroxide comparison experiments; and 10^5 cysts/mouse in the viability comparison, specific ozone residual and natural water experiments.

Pilot-scale experiments used the above procedure with the exception that an additional concentration step was required because the 1 L aliquot was centrifuged using six 175 mL plastic conical centrifuge tubes. The expected size of the inoculum was 10^4 cysts/mouse.

During experiments, mice were individually housed in filter-top cages (0.22 µm filter) in a SPF isolation room. The feces were checked daily for *G. muris* cysts from day 3 or 4 until day 8 or when they became positive. On day 8 necropsy was performed on all mice which were negative for *G. muris* cysts and the small intestine was examined for the presence of trophozoites.

A different procedure was used in some preliminary work (exps. 18 and 24). Disinfected liquid was centrifuged in a 50 mL plastic conical centrifuge tube at 800 x *g* for 10 min. The supernatant was removed, leaving a 0.5 mL centrifugate, which was split into a 0.100 mL portion for FDA-EB fluorogenic staining and a 0.4 mL portion for infecting mice. A minimum of 4 hemocytometer counts were made on the 0.4 mL portion in order to determine the total number of cysts. This portion was made up to 0.6 mL with Milli-Q® water. Each of 3 specific pathogen-free, male C3H/HeN mice were inoculated orally with 0.200 mL. The size of the inoculum was typically 10^4 cysts/mouse. All mice were caged separately and the feces checked for *G. muris* cysts every 2 d until day 20 or when they became positive.

4.3.8.2 *Giardia lamblia*

All disinfected liquid from the reactor was centrifuged in 175 mL plastic, conical centrifuge tubes at 800 x *g* for 10 min. The supernatant was removed, leaving a 1.5 mL centrifugate, of which 1.0 mL was used for infecting gerbils. Each one of a group of 5 Mongolian gerbils was inoculated orally with known numbers of *G. lamblia* cysts suspended in 0.2 mL of Milli-Q® water using techniques described previously (Belosevic and Faubert, 1983a; Belosevic *et al.*, 1983; Belosevic *et al.*, 1984). During experiments gerbils were individually housed in filter-top cages (0.22 µm filter) in a SPF isolation room. The total number of cysts released in a 4 h fecal collection (9:00 a.m. to 1:00 p.m.) by individual gerbils was determined for four consecutive days starting on day 10 postinfection. Cysts were isolated by sucrose gradient centrifugation and counted using a hemocytometer. On day 14 postinfection necropsy was performed on all gerbils, and the small intestine was examined for the presence of trophozoites (Belosevic *et al.*, 1983).

4.4 DISINFECTION PROCEDURES

4.4.1 Bench-Scale Experiments

Experiments with laboratory and natural waters used the same procedure with the exception that natural waters, with concomitant ozone demand, had a more concentrated stock ozone solution (up to 42 mg/L) added to them to minimize dilution effects. A 200 mL volume of test liquid was added to a 250 mL Erlenmeyer flask reactor. The test liquid was seeded with *Giardia* cysts to provide a concentration that was typically 10^4 cysts/mL for *G. muris* and slightly less (5×10^3 cysts/mL) for *G. lamblia*. The concentration of stock ozone solution was measured twice immediately before a calculated volume of ozone solution was added to the liquid using a mass-calibrated pipette. This was the applied ozone dose. Over the duration of the experiment, the test solution was pumped through a thermostatically controlled 35 µL, 1 cm light path length flow cell at approximately 8 mL/min. Residual ozone was neutralized using a slight excess of 1.0 M sodium formate. Sodium formate was also added to the controls. Temperature of the test liquid was maintained by means of an ice-water bath. Figure 4.2 depicts the experimental apparatus (p. 71).

In the screening experiment bentonite (technical grade; BDH) was added to the phosphate buffer to produce turbidity. In the viability comparison, specific ozone residual, natural water and *G. lamblia* experiments, Tween 20 (Sigma Chemical Co., St. Louis, Mo.) was added to the ozone reactor (0.01 percent vol/vol) after the ozone was neutralized to enhance cyst recovery. Following ozonation, but prior to concentration for the infectivity testing procedure, four replicate hemocytometer counts of cyst density were made. This was used to test the homogeneity of the cyst suspension and also permitted an accurate cyst "mass" balance as shown in Figure 4.4.

For the comparison of ozone and ozone–hydrogen peroxide, the procedure was similar to that described in the first paragraph of this section except that hydrogen peroxide (Perhydrol®; E. Merck, Germany) was added to the reactor to provide a final concentration of about 17 mg/L prior to adding cysts. The ozone dose was approximately 1.7 mg/L so that the theoretical hydrogen peroxide to ozone weight ratio was about 10 to 1. Residual hydrogen peroxide was neutralized using a slight excess of catalase. Hydrogen peroxide, sodium formate and catalase were used in the controls.

A different procedure was used in some preliminary work (exps. 18 and 24). A 50 mL reactor was filled with a calculated volume of ozone

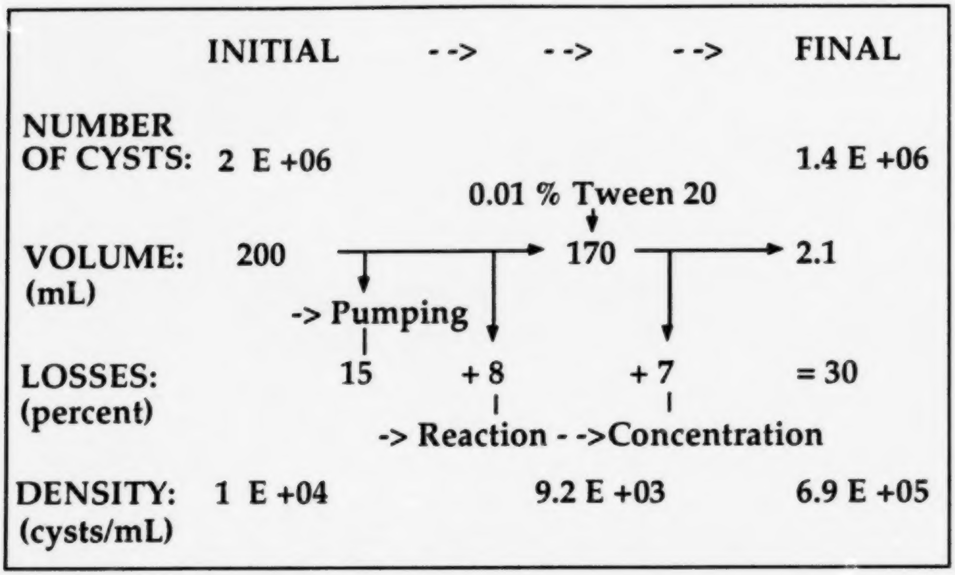

INITIAL
- ->
- ->
- ->
FINAL
NUMBER OF CYSTS:
2 E +06
1.4 E +06
0.01 % Tween 20
VOLUME: (mL)
200
170
2.1
-> Pumping
LOSSES: (percent)
15
+ 8
+ 7
= 30
-> Reaction - ->Concentration
DENSITY: (cysts/mL)
1 E +04
9.2 E +03
6.9 E +05

Figure 4.4 Cyst mass balance in control experiments

demand-free Milli-Q® water, *G. muris* cysts were added and then concentrated stock ozone solution was added to provide a total volume of 41 mL. The reactor was mixed rapidly (300 r.p.m.) using a magnetic stirrer (220T; Fischer Scientific) and Teflon®-coated stir bar during cyst addition, moderately during ozone addition (144 r.p.m.), and as slowly as possible (72 r.p.m.) for the 5 min contact time to minimize volatilization of the ozone. Ozone demand-free borosilicate glass pipettes or an Oxford pipette with ozone demand-free disposable tips were used for all liquid or ozone additions and for sample removals. The background u.v. absorbance (260 nm) of the cyst suspension was subtracted from the absorbance at the end of the contact time in order to determine ozone residual. Ozone was purged using 90 s of air-stripping and a final u.v. absorbance measurement was made.

4.4.2 Pilot-Scale Experiments

Pilot-scale experiments were performed using seeded *G. muris* cysts and MS2 coliphage concurrently. HPC bacteria that were native to the test water (NSR water) were enumerated as well. A total of 10^7 cysts were added to the 100 L reactor, providing an initial cyst density of approximately 10^2 cysts/mL. At predetermined times corresponding to the desired applied ozone doses, aliquots were extracted from the sampling port into 1 L Erlenmeyer flasks. These flasks contained 200 µL of 1.0 M sodium formate which neutralized the oxidant. Duplicate samples for ozone residual determination by the indigo method were taken immediately after the 1 L aliquots. As with bench-scale experiments, Tween 20 was added to enhance cyst recovery. Four replicate hemocytometer counts were made prior to concentration for the infectivity testing procedure. The control aliquot was obtained after *G. muris* cyst seeding but when the reactor was operating with oxygen prior to ozonation.

The feed-gas flow-rate was calibrated for standard temperature and pressure using a wet test meter (Precision Scientific, Inc.). Ozone concentration in the off-gas was continuously recorded on a strip chart with input from an u.v. spectrophotometer. The Rushton turbine was operated at 230 r.p.m. which corresponded to a gassed power input of 390 W/m^3 or a velocity gradient of 624/s. The applied ozone dose was calculated from (Finch and Smith, 1989):

$$C = \frac{C_g Q_g t_g}{V} \tag{4.1}$$

where: C is the applied ozone dose;

C_g concentration of ozone in the carrier gas;

Q_g is the gas flow-rate;

t_g is the ozonation time; and

V is the volume being treated (100 L).

4.5 EXPERIMENTAL DESIGN

Factorial experimental design permits the independent analysis of factors and their interactions using a minimum number of trials (Box *et al.*, 1978; Davies, 1979). Fractional factorial designs can be even more efficient, because negligible higher order interactions can be confounded with additional factors, resulting in fewer trials. A factorial design with two levels and n factors is designated as 2^n. A fractional factorial design is designated as 2^{n-m}, where m is the fraction, $1/2^m$, of the factorial. Factorial or fractional factorial designs were used wherever possible. The main effects and interactions were calculated from the regression coefficients (ß) by the relation:

$$\text{effect} = 2 \times ß \tag{4.2}$$

A method of analysis of variance (ANOVA) described by Davies (1979) was used to evaluate the factors and interactions. The mean square (MS) of each term was calculated as follows:

$$MS = (TE)^2/(2^{n-m}) \tag{4.3}$$

$$TE = ß \times 2 \times 2^{n-m-1} \tag{4.4}$$

where: TE is the total effect

An estimate of the experimental error permits discrimination of real from null effects. The mean square error (MS_d) is an estimate of the error variance and can be calculated from the mean square of insignificant effects and interactions. Half-normal probability plots provide a graphical technique for separating real effects from null effects (Daniel, 1959). The statistic MS/MS_d for each term may be compared with the *F*-statistic, $F\alpha(\lambda_1,\lambda_2)$, where α is the probability (*P*)-level, and λ_1 and λ_2 are the degrees of freedom for MS and MS_d, respectively. Those factors and interactions exceeding the *F*-statistic at the appropriate *P*-level are statistically significant. For most of the results reported here, the *P*-level selected was 0.05.

4.5.1 Comparison of Viability Methods

A single control trial and two or more ozonated trials were performed on each of four cyst preparations (A to D). Each control and ozonated trial had cysts concentrated from them with the resulting concentrate split three ways to provide cysts for the different viability determination methods. This design permitted a comparison of differences in the viability methods while eliminating run-to-run variations in the test water and ozonation conditions. One-way ANOVA was used to compare the three viability methods. The Duncan Multiple Range Test (Duncan, 1955) was used to determine if there was a significant difference between the viability methods.

4.5.2 Comparison of Ozone and Ozone–Hydrogen Peroxide

This experiment was designed as a series of paired trials using two different ozone demand-free laboratory buffer systems with the same cyst preparation. The first water was the 0.05 M phosphate buffer with hydrogen peroxide added in excess. A hydrogen peroxide to ozone weight ratio of 10 to 1 was used because it was found that this ratio completely decomposed ozone in 60 s or less, ensuring a large supply of decomposition radical species and little residual ozone (Yuen, 1990). Lower ratios were found to leave significant concentrations of residual ozone which would have confused the interpretation of the results. The second water was the 0.05 M phosphate-0.01 M bicarbonate buffer designed to quickly scavenge radical species from ozone decomposition.

The hypothesis being tested was that if the ozone–hydrogen peroxide process was more effective than ozone alone, the large quantity of radical intermediates produced in the peroxide system would result in more inactivation than ozone alone. All trials were conducted in triplicate at pH 5.7 and 22°C and with a 2 min contact time. Differences between mean inactivation in the two buffer systems was evaluated using a t-test.

4.5.3 Factors Affecting Ozone Inactivation

4.5.3.1 Screening experiment

This experiment was conducted to evaluate the significance of various water quality and process control parameters which have been reported to influence the ozone inactivation of *Giardia*. A 2^{5-1} fractional factorial experimental design was chosen to assess 5 factors in 16 runs (Box *et al.*, 1978). The factors and settings used were: applied ozone dose (1.5 and 3.0 mg/L); temperature (5 and 22°C); contact time (2 and 7 min); turbidity (0 and 5 NTU); and pH (5.7 and 7.6). In this type of design, no main effect or two-factor interaction is confounded with any other main effect or two-factor interaction (Box *et al.*, 1978). The experiment was designed in four blocks of four ozonated and two control trials each. In the design the CD and CE (contact time x turbidity and contact time x pH) interactions were confounded with block effects because these interactions were not expected to be significant. This permitted the detection of batch-to-batch differences in the cysts. Blocks and trials within blocks were conducted in random order.

It was assumed that the contact times and temperatures used in this experiment did not affect *G. muris* cyst viability permitting all control runs to be done at 22°C and for a contact time of 5 min. The effect of turbidity and pH on control cysts and, subsequently, the animal model, was not known, so these factors were varied in the controls.

4.5.3.2 Specific ozone residual experiment

This experiment was designed to further examine the effects of temperature and pH and to better define the kinetics of *Giardia* inactivation. Decomposition tests without cysts were used to calculate the applied ozone doses necessary to achieve a specific ozone residual level of approximately 0.4 mg/L in all trials (refer to Section 5.1.1). This is in contrast to the screening experiment where ozone dose was specified and the ozone residual was happenstance. Initially a full 2^2 factorial design with 2 centre-point replications was performed. Subsequently, additional trials were done in which contact time, temperature and pH were varied.

4.5.3.3 Natural waters experiment

This experiment was designed to verify the laboratory water results for two natural waters that were significantly different in water quality parameters affecting ozone decomposition. A 2^{3-1} fractional factorial design was performed using both NSR and GVRD waters. The factors and settings were: ozone residual (0.2 and 0.8 mg/L); temperature (5 and 22°C) and contact time (2 and 5 min). In addition, a 2^2 factorial design at 5°C was performed using GVRD water with factors and settings of: ozone residual (0.4 and 0.8 mg/L) and contact time (4 and 10 min).

4.5.4 Comparison of *G. muris* and *G. lamblia*

Four *G. lamblia* trials were performed: a control trial with a contact time of 5 min and ozonated trials with residuals of 0.2, 0.5 and 0.8 mg/L, and contact times of 2 and 5 min. A semiquantitative determination of *G. lamblia* inactivation was made. These results were compared qualitatively with *G. muris* inactivation results from other experimental trials made under similar ozonation conditions.

4.6 ANALYTICAL

The statistical application Data Desk Professional® v.3.0 (Odesta Corporation, Northbrook, Ill.) was used to perform least squares regression and routine statistical analyses. Systat® v.5.1 (Systat, Inc., Evanston, Ill.) was used to perform nonlinear analyses.

4.6.1 Fluorogenic Staining

The relation used for determining inactivation (N/N_O) from FDA-EB data was:

$$\frac{N}{N_o} = \frac{\text{% of stained cysts that are FDA-positive ozonated}}{\text{% of stained cysts that are FDA-positive control}} \qquad [4.5]$$

where: FDA-positive cysts are viable and have a green perimeter when viewed under u.v. light by using an epifluorescence microscope; and

EB-positive cysts are non-viable and fluoresce red by using epifluorescence microscopy.

4.6.2 Excystation

The equation used for determining percent excystation from *G. muris* excystation data was (Schaefer, 1990; USEPA, 1991):

$$\text{% excystation} = \frac{\text{ECW} + \text{PET}}{\text{ECW} + \text{PET} + \text{IC}} \times 100 \qquad [4.6]$$

where: ECW is the number of empty cyst walls;

PET is the number of partially excysted trophozoites; and

IC is the number of intact cysts.

Inactivation (N/N_O) was calculated from:

$$\frac{N}{N_o} = \frac{\text{% excystation ozonated}}{\text{% excystation control}} \qquad [4.7]$$

4.6.3 C3H/HeN Mouse Model

The relation used for determining inactivation (N/N_o) from mouse model data was:

$$\frac{N}{N_o} = \frac{n \times I}{n_o \times I_o} \qquad [4.8]$$

where: n is the number of positive mice in an ozonated trial;

I is the number of infectious cysts per positive mouse after ozonation, determined using the latent period (if the inoculum had 10^5, 10^4, 10^3, 10^2, or 10 infectious cysts, mice became positive on days 3, 4, 5, 6 or 8, respectively);

n_o is the number of positive mice in the control; and

I_o is the inoculum size in the control, determined by hemocytometer count.

In equation [4.8], the latent period definition is the time when all mice became positive. The term n was required because, on occasion, all mice in ozonated trials did not become positive. If all the mice in an ozonated trial did not become positive, the last day which showed an increase in the number of positive mice was used as the latent period. The term n_o was required in equation [4.8] because an occasional mouse in the ozonated cohort died before day 8 and it was necessary to use the same number of animals in the control cohort to estimate the initial number of viable cysts. An example of this calculation is provided in Appendix B.

4.7 QUALITY ASSURANCE AND QUALITY CONTROL

4.7.1 Biological

The most important component of QA/QC in *Giardia* work deals with maintaining SPF colonies of mice and gerbils. It is well established that prior exposure of animals to either *G. muris* or *G. lamblia* can lead to resistance to reinfection (Roberts-Thomson *et al.*, 1976b; Belosevic and Faubert, 1983b; Belosevic *et al.*, 1983; Lewis *et al.*, 1987). Thus it is imperative that all animals be maintained in a SPF facility before experimental use. Mice and gerbils were purchased from SPF facilities and were kept under SPF conditions (laminar flow isolator) upon arrival. Access to the laminar flow isolator was limited to two animal-care technicians. Animals entering the laboratory were treated for three consecutive days with Metronidazole (Flagyl®; Rhone-Poulenc) to ensure that they were free from all possible protozoan infections (Belosevic and Faubert, 1983a; 1983b; Belosevic *et al.*, 1983). Fecal examinations as well as small intestine examinations for trophozoites of animals chosen at random were performed one week after treatment.

Giardia determinations were performed using groups of five mice for each experimental trial including controls. Invariably C3H/HeN mouse control groups became positive on day 3 or day 4 depending on the size of the inoculum, indicating that *G. muris* cyst preparations were of high quality and the experimental protocol was satisfactory. QA/QC measures for *G. muris* demand-free work included:

1. the absorbance of a cyst suspension of 10^4 cysts/mL at 260 nm was never greater than 0.03/cm;
2. cysts were stored at 4°C for 18 h and were used within 48 h of preparation.

Only the absorbance QA/QC measure was applicable to *G. lamblia* demand-free work.

The underlying probability distribution of replicate hemocytometer cyst count type data is the Poisson distribution (Fisher *et al.*, 1922). The Fisher D^2 statistic and four replicate hemocytometer counts of the cyst density following the ozonation reaction were used to test the homogeneity of the cyst suspension. If the variation among replicates exceeded that from chance alone ($P \leq 0.05$) the data were discarded. An example of this test is provided in Appendix C. It was not necessary to discard any data due to this criterion.

A critical aspect of QA/QC was liaison between the environmental engineering laboratory where ozonation was done and the zoology laboratory where infectivity testing was performed. A written plan for each experiment was prepared in advance. Forms were developed to facilitate the recording of data and communication between laboratories.

4.7.2 Chemical

Reagents which came into contact with ozone were prepared using high-purity chemicals (AnalaR grade; BDH). The quality of reagents had an extremely important effect on ozone decomposition.

Aqueous residual ozone was determined using u.v. absorbance. The indigo trisulfonate method (APHA *et al.*, 1989) was periodically used as a check. The two methods agreed within 5 percent, however the indigo method was consistently higher due to the different molar absorption coefficient (2,950 M^{-1} cm^{-1}) used to initially calibrate the procedure (Bader and Hoingé, 1981). Typical results are given in Appendix D.

4.7.3 Instrumentation

Although iodometric methods for ozone determination may proceed through a variety of pathways resulting in non-constant stoichiometry, they are useful as an independent check of gas phase u.v. monitors (Gordon *et al.*, 1989). The gas phase u.v. monitor was checked on a monthly basis using an iodometric method recommended by the manufacturer (Birdsall *et al.*, 1952). Spectrophotometers were checked monthly with solutions of known absorbances in the visible and u.v. wavelength regions. Other instruments and equipment were maintained and checked in accordance with manufacturers' instructions.

5. RESULTS AND DISCUSSION

5.1 PRELIMINARY RESULTS

5.1.1 Ozone Decomposition

Decomposition kinetics of ozone in the laboratory waters were determined using the u.v.-visible diode-array spectrophotometer and the bench-scale disinfection procedure without cyst addition. Trials were carried out in a darkened room and reactors were mixed at 72 r.p.m. using a Teflon®-coated stir bar and a magnetic stirrer (220T; Fischer Scientific). Experimental conditions for Data Set A trials were 22°C and pH 6.9. Conditions for Data Set B trials were as noted in Appendix E.

Data Set A was developed to study the combinations of ozone reactor, applied ozone dose and contact time that would provide ozone residuals in the range necessary to achieve 3 log inactivation of *Giardia*. A replicated complete factorial design was used to examine 3 factors in 24 trials (Table E.1, Appendix E). The factors and settings used were: size of ozone reactor (50, 250 and 2,000 mL); applied ozone dose (0.5 and 1.0 mg/L); and type of laboratory water (ozone demand-free phosphate buffer and ozone demand-free deionized water). The ozone half-lives that could be achieved in the 250 mL Erlynmeyer reactor were 15–20 min for phosphate buffer and 30–60 min for deionized water. A 15–20 min ozone half-life is significant in terms of the contact times which would normally be considered in engineering applications for disinfection (10 min or less). An advantage of the 250 mL Erlynmeyer reactor compared with the 2 L vessel is that six to eight trials can be done with the same quantity of cysts. A disadvantage of the 250 mL Erlynmeyer reactor is potential volatilization; however, with a test liquid volume of 200 mL, the surface area exposed to the atmosphere is minimal. An ANOVA using mean half-lives from Data Set A is given in Table E.2 in Appendix E. The significant factors were type of laboratory water and size of ozone reactor ($P \leq 0.05$). Interestingly, ozone decomposition was independent of the applied ozone dose, which indicated that ozone decomposition generally appeared to be a first-order reaction. This conclusion was verified by plotting decomposition values as log C/C_o versus time and as $[(1/C) - (1/C_o)]$ versus time. The first-order plot (log C/C_o vs time) was linear in 21 trials while the second-order plot appeared linear in 3 trials.

Data Set B was used to predict the applied ozone doses necessary to obtain the desired residuals in the specific ozone residual experiment. Data Set B was a 2^4 factorial designed to assess 4 factors in 16 runs. The factors and settings used were: applied ozone dose (1.5 and 3.0 mg/L); temperature (5 and 22°C); turbidity (0 and 5 NTU); and pH (5.7 and 7.6). This was similar to the screening experiment design. Work was done at the same time for quality control. Figures 5.1–5.4 show the behavior of ozone in the phosphate buffer and 250 mL Erlynmeyer reactor (Data Set B, Appendix E). In Figures 5.1–5.4 ozone decomposition appears independent of the applied ozone dose and the linearity of the log C/C_o versus time plots also indicates first-order decomposition. Decomposition results at each pH value were repeatable. The regression equation for the first-order decomposition coefficient was:

$$k = [\, 6.54 + 1.80(\text{pH}) + 0.34(\text{Temp}) - 0.31(\text{pH})(\text{Temp})\,]\, 10^{-4} \qquad [5.1]$$

where: (pH) assumes a value of -1 for pH 5.7 and +1 for pH 7.6;

(Temp) assumes a value of -1 for 5°C and +1 for 22°C; and

k is the first-order decomposition coefficient in s^{-1}.

C_o was calculated using k from equation [5.1] and the relation:

$$C_o = C_r/10^{-kt} \qquad [5.2]$$

where: t is the contact time in seconds;

C_r is the ozone residual in mg/L; and

C_o is the applied ozone dose in mg/L.

Tables E.3 and E.4 in Appendix E summarize the regression and indicate the significance of coefficients in equation [5.1]. Turbidity did not significantly affect ozone decomposition in Data Set B.

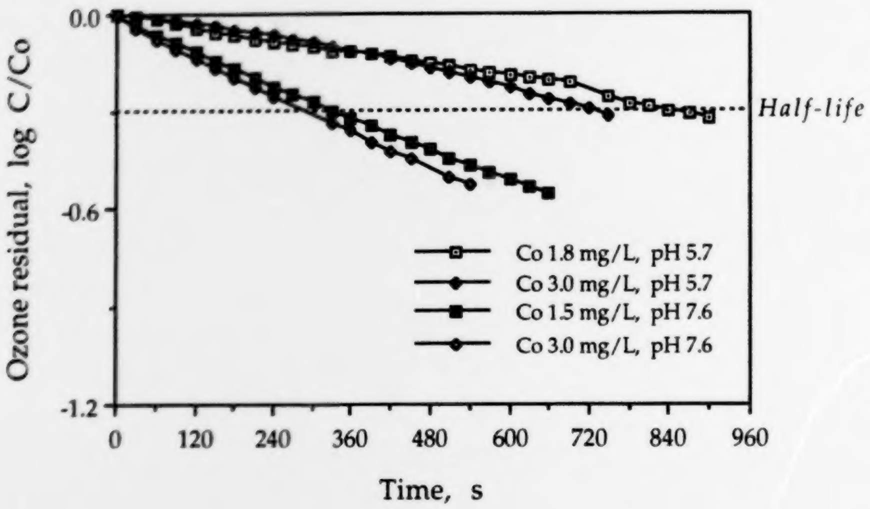


Figure 5.1 Ozone decomposition in 0.05 M phosphate buffer and 250 mL Erlynmeyer reactor (5°C, 0 NTU)

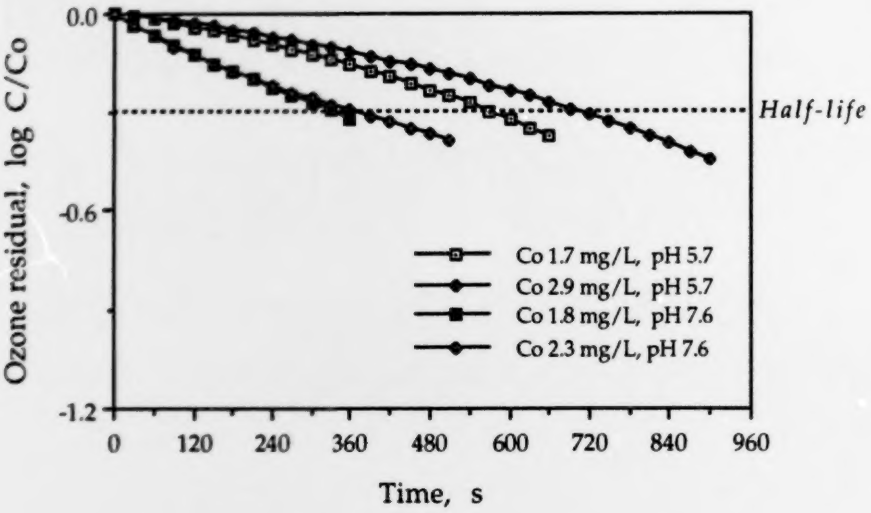


Figure 5.2 Ozone decomposition in 0.05 M phosphate buffer and 250 mL Erlynmeyer reactor (22°C, 0 NTU)

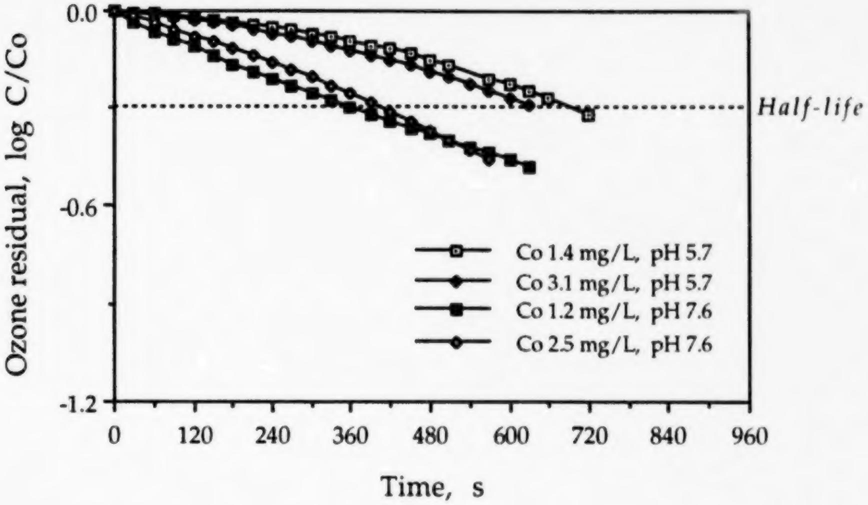


Figure 5.3 Ozone decomposition in 0.05 M phosphate buffer and 250 mL Erlynmeyer reactor (5°C, 5 NTU)

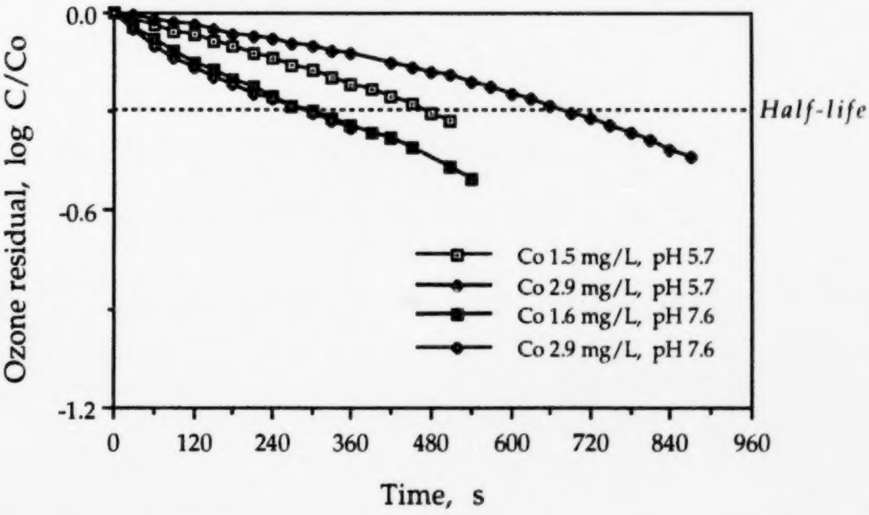


Figure 5.4 Ozone decomposition in 0.05 M phosphate buffer and 250 mL Erlynmeyer reactor (22°C, 5 NTU)

5.1.2 Verification of Mouse Model

The mouse model was originally described by Iwanczuk (1968) and Roberts-Thomson *et al.* (1976a) and the correlation between oral dosage and course of infection was further defined by Belosevic and Faubert (1983a). The results reported in 1983 were for CD-1 Swiss mice; therefore, it was necessary to verify the results for male C3H/HeN mice. Data verifying the effect of different cyst doses on the latent period in SPF male C3H/HeN mice are presented in Table 4.2 (p. 80). Further replications of the effect of different doses of cysts on the time until all mice in a group released cysts in the feces are found in the control trial animal responses in Appendices G and H. Refer to trial C1 in Table H.2 for the 10^3 cyst dose; control trials in Tables H.1 and H.2 for the 10^4 cyst dose (11 replications); and control trials in Table G.3, H.1 and H.3 for the 10^5 cyst dose (10 replications). In each of these control trials, all mice in the group were positive on day 3 for the 10^5 cyst dose; day 4 for the 10^4 cyst dose; and day 5 for the 10^3 cyst dose. Not only were all control mice groups positive, but groups invariably were positive with a latent period that depended on the inoculum size.

An additional experiment was designed to replicate animal responses to 10^2, 10 and 1 cyst doses. All mice in a group of 9 became positive on day 6 for the 10^2 cyst dose. Five mice in a group of 5 became positive on day 8 for the 10 cyst dose. These latent periods agree with those presented in Table 4.2. However, none of the mice in the 1 cyst dose group were positive for cysts on day 10 postinfection, nor were trophozoites detected on day 12. This result was not obtained previously. Consequently the C3H/HeN mouse-*G. muris* model was not used in the range of 10 or less cysts. Latent periods of 3 days for a 5×10^4 cyst dose and 5 days for a 10^3 cyst dose were reported by Schupp and Erlandsen (1987b) for non-Swiss CF-1 mice and are similar to those reported here for C3H/HeN mice (Table 4.2).

Equation [4.8] (p. 92) is an empirical relation based on observed experimental results rather than a theoretical dose-response relation. In equation [4.8], the latent period is used to assess infectivity. The effect of different doses of *G. muris* cysts on the latent period is shown in Figure 5.5. Although a quantal (infected or not infected) response was observed in mice exposed to ozone treated cysts, the classical ID_{50} technique (Klaassen *et al.*, 1986) was not appropriate for assessing infectivity since as few as one *G. muris* cyst was an infectious dose to 9 of a group of 12 inbred male C3H/HeN mice. Advantages of the latent period approach include the need for just a single cohort of mice and the avoidance of an assumption regarding the probability of infection as in most probable number based methods (Haas and Heller, 1990).

Tween 20 increased cyst recovery in control trials from 6.2 ± 1.6 percent to 69 ± 2.8 percent (mean ± SD). Consequently, the inoculum size was large and it was necessary to collect data on day 3 to properly use the C3H/HeN mouse model. Tween 20 was used in bench- and pilot-scale experiments as noted in Sections 4.4.1 and 4.4.2. Tween 20 also increased recovery in the ozonated trials, however interpretation was complicated by variable experimental conditions and ozonation levels as well as the suspected lysing of cysts. For a variety of reasons including cyst clumping, declining ozone residual during the experiment, possible nonuniformity in the action of ozone, or simply high inactivation, not all mice in an ozonated trial group may become infected. When this occurs, it can be concluded that more cyst inactivation was obtained than if all mice in the group had been infected. The relation for determining N/N_0 from infectivity data [equation 4.8] was formulated on this basis.

In-depth analysis of sources of variance in the mouse model was beyond the scope of this work, however, a number of observations can be made. Sampling was not a major source of error in the mouse model since typically 60 percent of the initial cyst population was being assessed. However, because mice were only checked daily, the C3H/HeN mouse model must be considered a discontinuous rather than a continuous function. Many inactivations were separated by a whole number (i.e. 1.2, 2.2 etc.). This was because a 1, 2 or 3 day lag in the latent period of the ozonated trial relative to the control was indicative of 1, 2 or 3 log inactivation. The impact on the accuracy of the mouse model is a potential overestimation of inactivation to approximately 3 log-units.

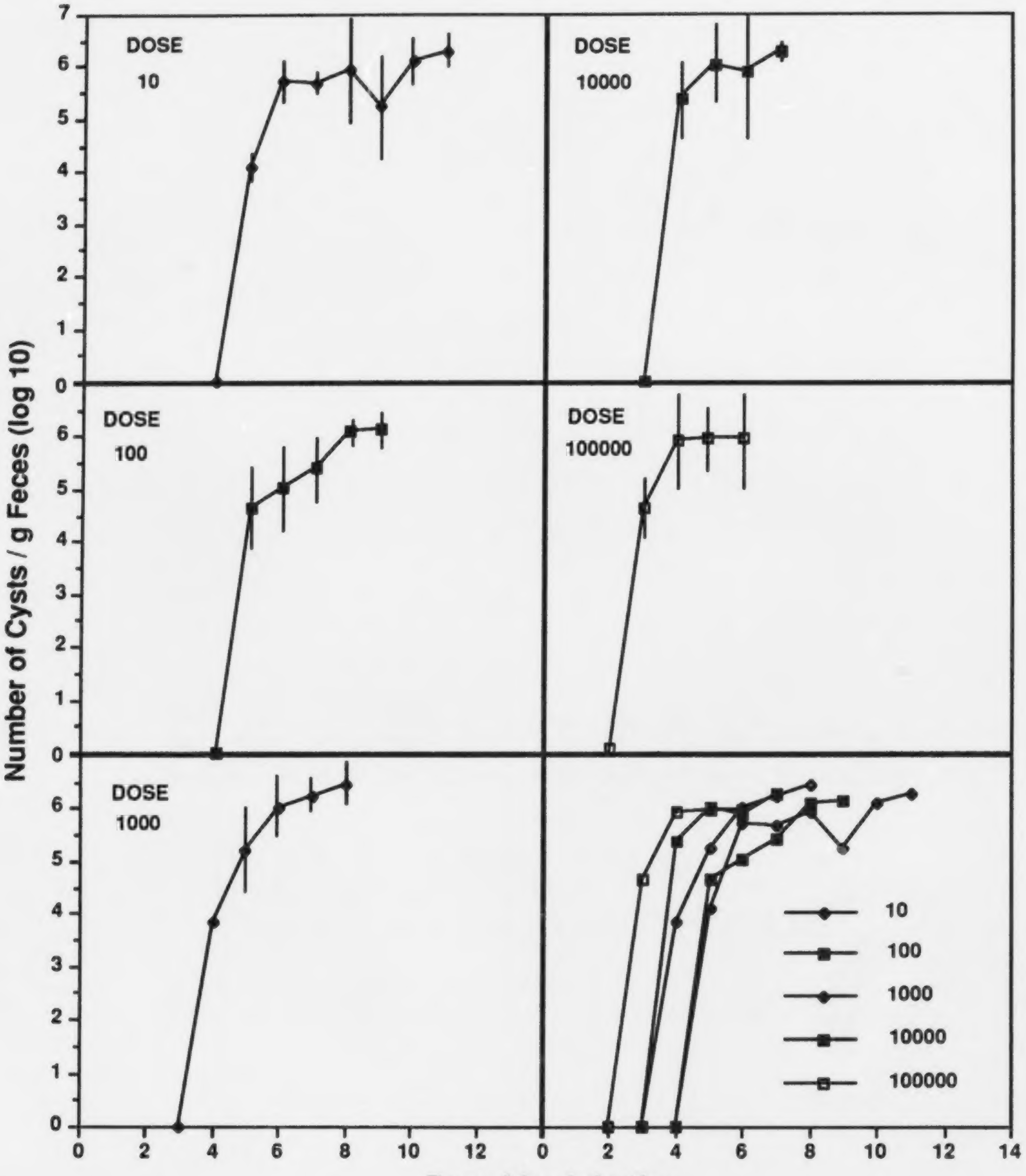


Figure 5.5 Pattern of cyst release in C3H/HeN mice infected with different doses of *Giardia muris* cysts. Each point represents the mean number of cysts released for 10 animals ± SD.

5.2 COMPARISON OF VIABILITY METHODS

5.2.1 Objective

The objective of this experiment was to compare *G. muris* cyst inactivation in laboratory water at bench-scale following ozonation using three viability methods on split samples. The methods used were a FDA-EB staining technique, the C3H/HeN mouse-*G. muris* model, and *in vitro* excystation.

5.2.2 General Discussion

Experimental conditions and inactivation results for the three viability methods are summarized in Table 5.1. Details on experimental conditions, hemocytometer count data and animal responses are presented in Appendices F and G (Tables F.1, G.1, G.2 and G.3).

The final centrifugate in controls contained approximately 1.4×10^{6} *G. muris* cysts in 2.1 mL. Mean recovery of control cysts was 68 ± 9 percent (mean ± SD). Cyst losses totalled approximately 30 percent with the greatest loss (15 percent) accounted for by pumping during the reaction. Excystation values in controls were 96.3, 99.1, 95.8 and 93.0 percent for preparations A, B, C and D, respectively, as indicated in Figure 5.6. The mean control excystation was 96.1 ± 2.5 percent, indicating that cyst preparations were of high quality. Following fluorogenic staining three populations of cysts were observed: green (FDA-positive), red (EB-positive), and black (unstained). It was easier to observe black cysts under bright-field than with epifluorescence microscopy. The fraction of control cysts that were nonviable or EB-positive ranged from 9 to 12 percent (Figure 5.6). Forty to 60 percent of *G. muris* cysts in controls were unstained. However this fraction decreased to 6.9 ± 5.5 percent following ozonation, with a range of 1.9 to 16.1 percent. Figure 5.7 summarizes the effect of ozone on fluorogenic staining results.

Table 5.1 Experimental conditions and bench-scale ozone inactivations estimated by three viability methods for *G. muris* in ozone demand-free phosphate buffer (pH 6.7; 22°C).

Trial	Contact time (min)	Residual ozone (mg/L)	Inactivation (log N/N_0)†		
			FDA-EB staining	*In vitro* excystation	C3H/HeN mouse model
A1	5	0.3	>2.1	>2.8	4.2
A2	5	0.4	>2.2	>2.7	4.2
B1	5	0.1	2.3	3.0	3.1
B2	5	0.1	2.7	>3.0	3.1
C1	5	1.3	>2.8	>2.9	4.4
C2	0.58	0.04	0.2	0.5	1.2
C3	0.25	0.2	0.006	0.7	ND
C4	0.33	0.1	0.004	0.2	ND
D1	0.58	0.2	0.5	1.8	2.2
D2	1	0.1	0.7	2.1	ND
D3	2	0.1	2.4	2.0	ND
D4	1.53	0.02	0.5	2.4	ND

† >, detection limit;
FDA, fluorescein diacetate;
EB, ethidium bromide;
ND, not determined.

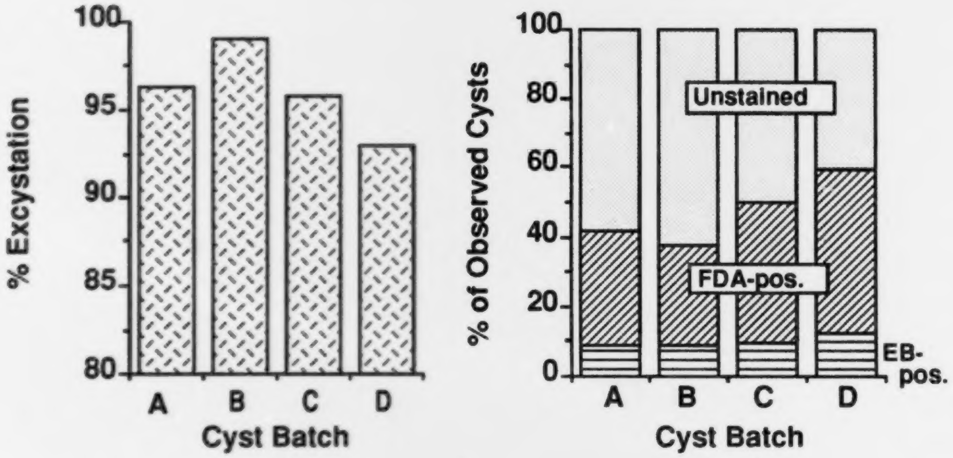


Figure 5.6 Excystation and fluorogenic staining in control *G. muris* cysts in the viability comparison experiment

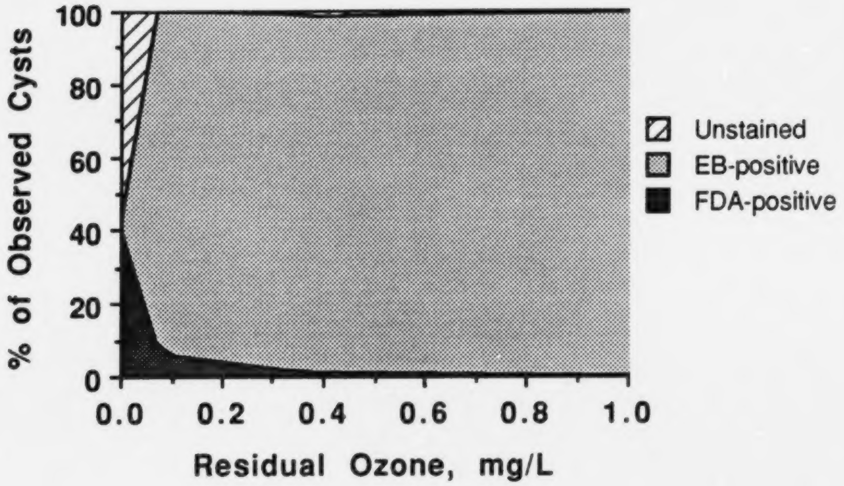


Figure 5.7 Effect of ozone on *G. muris* cyst staining (22°C, pH 6.9, 300 s contact time)

Trials A1, A2 and C1 had ozone residuals of 0.3 mg/L or higher. Although the entire slide area was examined in the excystation method for these trials, it was only possible to count 669, 546 and 782 cysts, respectively, with all cysts being intact cysts. The corresponding counts in the fluorogenic dye method were 143, 169 and 675, with all cysts being either EB-positive or unstained. These trials were at the detection limit since there was neither positive excystation, nor were FDA-positive cysts observed. None of the mouse model results were at the detection limit since at least one mouse became positive in each of the trials.

The three viability methods were compared using one-way ANOVA. Results are summarized in Table 5.2. In the analysis, trials A1, A2 and C1 were excluded because they were beyond the detection limit of all methods except infectivity. Fluorogenic staining was used as a reference method because the difference between all of the other methods could be calculated. The ANOVA was performed by using differences from the reference method. The underlying assumptions of ANOVA were tested and found to be satisfied. The Duncan multiple range test was used to determine if there was a significant difference between the viability methods (Duncan, 1955). Fluorogenic staining significantly underestimated inactivations determined by using infectivity ($P \leq 0.05$). This would suggest that not all FDA-positive cysts were capable of producing an infection. In contrast, the difference between infectivity and excystation, and between fluorogenic staining and excystation was not statistically significant ($P \leq 0.05$).

Table 5.1 indicates that fluorogenic staining gave the highest viabilities, or smallest inactivations, and infectivity provided the lowest viabilities. Excystation provided results intermediate to the other two methods. There was a greater difference between fluorogenic staining and the other methods at lower inactivations than at higher ones, as can be seen by comparing those trials which had an ozone residual of 0.1 to 0.2 mg/L. However, the animal model is a measure of the potential of the cysts to complete their life cycle in the host. In contrast, fluorogenic staining measures the presence or absence of metabolic activity. It is possible that cysts which show some metabolic potential

Table 5.2 One-way ANOVA for three viability determination methods performed on split-samples following ozone inactivation of *G. muris*.

Source	Sum of squares	Degrees of freedom	Mean square	*F*-ratio	Probability
Between viability methods	1.97	2	0.986	6.005	0.022
Error	1.48	9	0.164		
Total	3.45	11			

will not cause an infection in the animal host, thereby causing an apparent overestimation of viability.

A qualitative comparison of the three viability methods in terms of basis of the method, equipment, time required, and theoretical and observed detection limits following ozonation is provided in Table 5.3. Observed detection limits did not always equal their theoretical values and infectivity required considerably more time than the other methods. The time given in Table 5.3 for the mouse model was that required for a test trial latent period of up to five days greater than that of the control. To demonstrate 3 log inactivation, it would only be necessary to check control mice on day 3 and test mice on day 6. In the numerical comparison of inactivation estimates following ozonation, it should be remembered that the basis of each viability method, as indicated in Table 5.3, is different. The fact that two *in vitro* methods are being compared with one *in vivo* method adds an additional dimension of uncertainty.

Observed fluorogenic staining and *in vitro* excystation detection limits were 2.1 to 3.0 log inactivation depending on the number of cysts counted or found. In three of the twelve excystation trials, there were less than 1,000 cysts available for counting. FDA-EB inactivation estimates were based on stained cysts only, and the number counted ranged from 140 to 966 in the 12 trials. The theoretical detection limit for both of these methods is 3.0 log inactivation based on the microscopic examination of 1,000 cysts. The theoretical detection limit for the C3H/HeN mouse-*G. muris* model is based on the size of the inoculum in the control (10^5 cysts) and is 5 log inactivation. The observed mouse model detection limit was greater than 4.4 log inactivation.

The relation for determining N/N_O from infectivity data [equation 4.8] was formulated to provide higher inactivations when fewer mice became positive. An example is trials C1 and A1, with 4.4 and 4.2 log inactivation, respectively. In trial C1 there was only one positive mouse on day 8 and the ozone residual was 1.3 mg/L. In trial A1 there were four positive mice on day 8 and the ozone residual was 0.3 mg/L. Trial C1 had one-fourth the number of positive mice compared

Table 5.3 Generic comparison of three viability methods in terms of basis of method, equipment and time required, and theoretical and observed detection limits following ozonation.

		Detection limit (log N/N_0)			
Viability method	Basis of method	Theoretical	Observed	Time required	Specialized equipment
FDA-EB	Biochemical metabolism or exclusion	3.0	2.1 to 2.8	1 h	Epifluorescent microscope
In vitro excystation	Chemical induction	3.0	2.7 to 3.0	3 h	Phase contrast microscope
C3H/HeN mouse model	Measure of infectivity	5.0	> 4.4	8 d	Animal care facilities

FDA, fluorescein diacetate;
EB, ethidium bromide.

with trial A1 at an ozone residual that was about four times as high.

5.2.2.1 Fluorogenic staining

A potential problem with fluorogenic dyes is that they are sometimes affected by the particular disinfectant used (Sauch *et al.*, 1991). This did not appear to be the case with ozone and FDA-EB. Unstained cysts were not used in calculating inactivation because their numbers decreased markedly following ozonation, and assumptions would have been required regarding unstained cyst viability. Schupp and Erlandsen (1987b) found that unstained *G. muris* cysts were infective to mice in a study that did not involve disinfectants. Other researchers found that 21 percent of *G. lamblia* cysts were unstained with FDA-EB after 30 min of exposure to 1 mg/L of chlorine (Hale *et al.*, 1985). Unstained cysts decrease the efficiency of fluorogenic staining procedures in determining inactivation because 1,000 stained cysts rather than 1,000 total cysts must be counted to determine 99.9 percent inactivation.

5.2.2.2 Excystation

In vitro excystation is based on chemical induction. Care must be taken to determine whether the excysted trophozoites are alive or dead in order to avoid counting aborted attempts at trophozoite emergence (Schaefer, 1990; Schupp and Erlandsen, 1987b). Results from excystation replicates B1 and B2 agreed even though one of the values was at the detection limit. In trials A1, A2 and C1, which had ozone residuals of 0.3 mg/L or higher, it was not possible to find 1,000 cysts using the *in vitro* excystation procedure. It was suspected that one of the modes of action of ozone was to lyse cysts, because all factors except ozone residual were the same as in other trials where 1,000 cysts were found.

5.2.2.3 Infectivity

Animal infectivity provided a direct measure of the ability of cysts to cause infection. Infectivity provided information on groups of cysts rather than on individual cysts, and consequently detected high inactivations. No other viability method was capable of detecting inactivations with ozone residuals of 0.3 mg/L or higher in the demand-free phosphate buffer. C3H/HeN mouse model replicate trials B1 and B2 both had an inactivation of 3.1 log-unit. Using the error variance found in Table 5.2 and the appropriate t-statistic, the 95 percent confidence limit for the mean inactivation in trials B1 and B2 was:

$$\bar{x} \pm \frac{2.26\sqrt{0.164}}{\sqrt{2}} \text{ or } \bar{x} \pm 0.65 \text{ log}$$

Alternative infectivity methods to that used in this work include the ID_{50} technique and the most probable number method. Although ID_{50} has superior statistical properties, it was not used because the latent period approach avoided the expense of a cohort of mice at each dilution level, as required with the ID_{50} and most probable number methods.

5.2.2.4 Comparison with other studies

DeRegnier *et al.* (1989) noted that all *Giardia* cysts capable of producing infection would show viability by fluorogenic dyes or excystation, but not *vice versa.* Inspection of Table 5.1 (p. 103) reveals that generally infectivity indicated the highest inactivations, followed by *in vitro* excystation, and inactivation estimates from fluorogenic staining were the lowest. Smith and Smith (1989), working with *G. lamblia* reported that FDA staining consistently overestimated cyst viability compared with *in vitro* excystation. This result is in agreement with the results reported here for *G. muris* where lower FDA-EB inactivations were indicated in Table 5.1 except for one trial where excystation was lower. It is also consistent with the results of Ongerth *et al.* (1989), who reported that fluorogenic staining indicated a slightly higher proportion of viable *G. muris* cysts than did *in vitro* excystation after heat inactivation.

Based on very limited replication, the 95 percent confidence limit was ± 0.65 log-units for *G. muris* inactivations determined by the mouse model. This confidence limit is also applicable to the two other viability methods because it was calculated using a pooled error variance. For comparison, the 95 percent confidence limit for *E. coli* in ozone demand-free solutions has been reported as ± 0.5 log-unit in the 3 to 7 log inactivation range (Finch *et al.*, 1988). It is expected that sampling may be a major source of error in the fluorogenic staining and *in vitro* excystation methods. An estimate of sampling variance can be made using sampling theory (Cochran, 1977). The sample size n was nominally 10^3 for FDA-EB and excystation. The population size N was 6.9×10^4 for FDA-EB and 3.5×10^5 for excystation in trial B1. The 95 percent confidence limit due to sampling is ± 0.3 log for trial B1 as estimated by FDA-EB and ± 0.6 log for trial B1 as estimated by excystation.

Previous studies comparing excystation and infectivity following chemical disinfection are rare. Wallis *et al.* (1990) reported $Ct_{99.9}$ values for *G. muris* cyst ozonation of 1.6 mg·min/L as estimated by excystation and 1.1 mg·min/L as estimated by animal infectivity. Animal results were interpolated from graphs at points where a mixed infection of five animals occurred. Another study showed little difference between infectivity and excystation up to 99.9 percent inactivation when using *G. muris* cysts and an ID_{50} chlorination technique (Hoff *et al.*, 1985). The results of this work by using a course of infection based animal model and ozonation support the hypothesis that *in vitro* excystation and infectivity provide similar results when using *G. muris* cysts. However, in the present work, only animal infectivity had the sensitivity to detect inactivations greater than 99.9 percent. Because of its high sensitivity, animal infectivity was used exclusively in further experimental work in this research.

5.3 COMPARISON OF OZONE AND OZONE–HYDROGEN PEROXIDE

5.3.1 Objective

The objective of this experiment was to distinguish between the disinfection effects of the ozone molecule alone and that of ozone decomposition products when inactivating *Giardia* cysts. The bench-scale disinfection procedure was used in conjunction with the C3H/HeN mouse-*G. muris* model.

5.3.2 Discussion

Figures 5.8 and 5.9 show ozone concentration versus time for the three trials using each laboratory buffer system. Figure 5.9 clearly indicates the beneficial effects of bicarbonate in quenching the ozone decomposition reaction. Inactivation results are presented in Figure 5.10. Further details on experimental conditions and animal responses can be found in Appendices F (Table F.3, exp. 31 data) and H (Table H.1).

The mean *G. muris* cyst inactivation in the phosphate-bicarbonate buffer system was 3.9 ± 0.9 log inactivation (mean ± SD) compared with 2.3 ± 0.2 log inactivation in the hydrogen peroxide system. The phosphate-bicarbonate buffer system, which had little decomposition of ozone over the duration of the experiment (mean residual ozone of 1.5 ± 0.1 mg/L at 2 min which was 92 ± 2 percent of the applied ozone dose), had significantly greater ($P \leq 0.05$) inactivation of *G. muris* cysts than that observed in the phosphate buffer hydrogen peroxide system where the ozone was completely decomposed in less than 60 s.

The hydroxyl radical is extremely reactive and very short-lived in aqueous solutions, so it is most likely to react with molecules of dissolved species, which have greater surface area to volume ratios than the larger microorganisms. By inference, the results reported here indicated that the direct action of the ozone molecule appeared to be the most significant factor for inactivation of *G. muris.* The 2.3 log inactivation in the peroxide system can be attributed to the average ozone residual (0.75 mg/L) that was present over the 60 s it took the ozone to decompose. This amount of ozone is sufficient to inactivate at least 99 percent of the *G. muris* cysts in 60 s and is 150 times greater than the ozone residual required to inactivate 99.9 percent of *E. coli* in 60 s in ozone demand-free solutions (Finch *et al.*, 1988).

Researchers working with *B. cereus* and *G. muris* concluded that ozone–hydrogen peroxide was applicable for disinfection but was less efficient than ozone alone, particularly at higher hydrogen peroxide to ozone weight ratios (Duget *et al.*, 1989; Wolfe *et al.*, 1989b). Observations

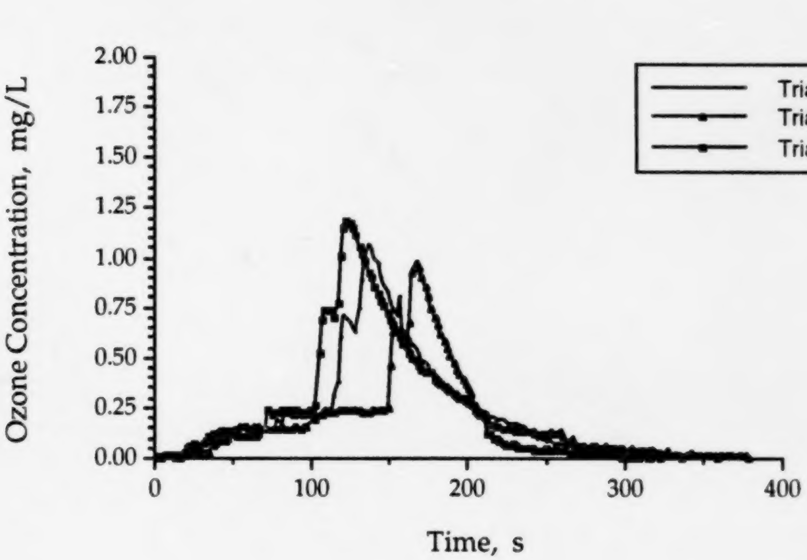


Figure 5.8 Ozone decomposition in 0.05 M phosphate buffer with a 10 to 1 hydrogen peroxide to ozone weight ratio.

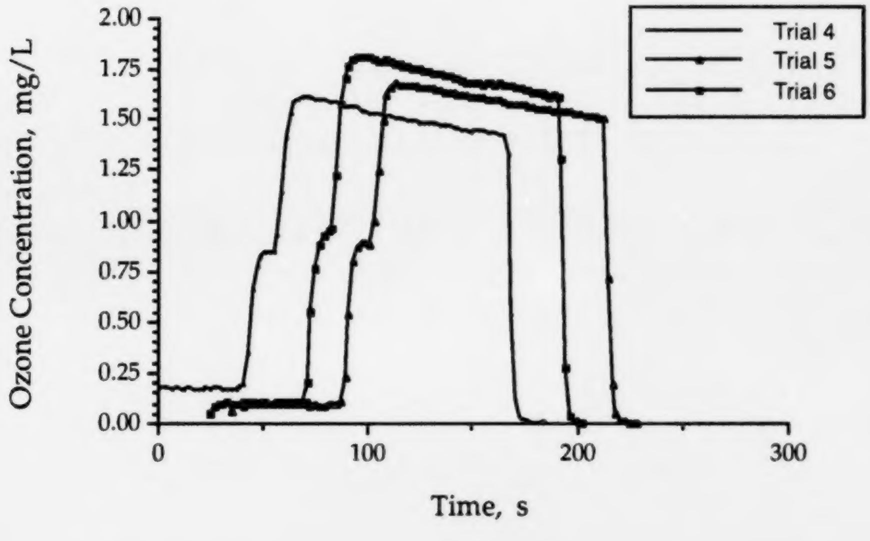


Figure 5.9 Ozone decomposition in 0.05 M phosphate-0.01 M carbonate buffer.

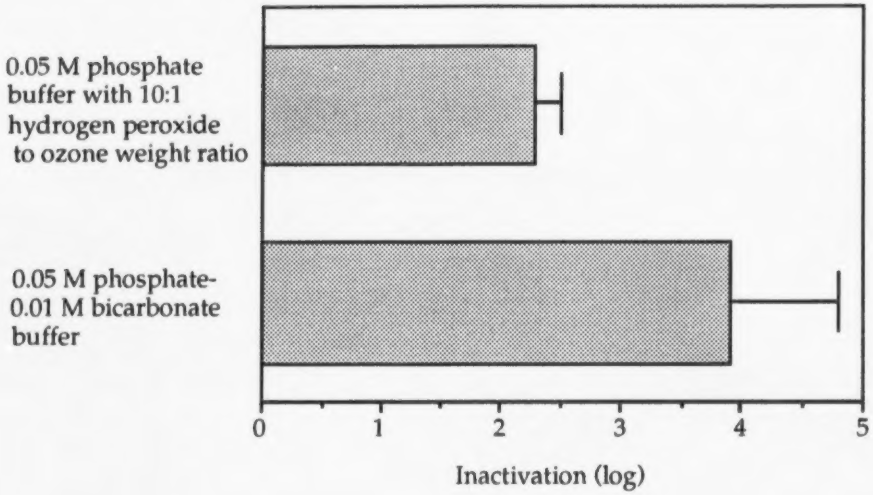


Figure 5.10 Mean *G. muris* cyst inactivation (mean of three trials plus one standard deviation) in two different laboratory buffer systems

made with *E. coli* and two buffer systems similar to those reported here could be explained either in terms of ozone decomposition products or the ozone molecule; however, inactivation continued in the presence of an ozone residual and not in the phosphate buffer hydrogen peroxide system (Yuen, 1990; Finch *et al.*, 1992). These findings in conjunction with the work reported here suggest that ozone alone is a more effective disinfectant. The ozone–hydrogen peroxide process was not researched further because there did not appear to be a significant disinfection effect associated with the hydroxyl radical reaction pathway.

5.4 FACTORS AFFECTING OZONE INACTIVATION

5.4.1 Objective

The objective of this experiment was to systematically evaluate the significance of parameters including temperature, turbidity, pH, ozone concentration and contact time in the ozone inactivation of *G. muris*. The bench-scale disinfection procedure was used in conjunction with the C3H/HeN mouse-*G. muris* model. Phosphate buffer was used in the screening and specific ozone residual experiments while NSR and GVRD waters were used in the natural waters experiment.

5.4.2 General Discussion

Experimental conditions and inactivation results for the screening, specific ozone residual and natural waters experiments are summarized in Table 5.4, 5.5 and 5.6, respectively. Further details on experimental conditions can be found in Appendix F (Table F.2, exp. 30 data for screening; Table F.3, exps. 36 and 41 data for specific ozone residual; Table F.4, exp. 39 data for natural waters). Animal responses are presented in Appendix H (Tables H.2, H.3 and H.4).

5.4.2.1 Screening experiment

Inactivation results, calculated using equation [4.8] (p.92) are shown in the last column of Table 5.4(a). In 4 of the 20 test trials no mice

Table 5.4(a) Screening Experiment: Design and bench-scale ozone inactivation results for *G. muris* in ozone demand-free phosphate buffer.

		Factor					Inactivation, $\log\frac{N}{N_o}$
Block	Trial	A	B	C	D	E	
1	5	-	-	+	-	-	3.5
	8	+	+	+	-	-	3.2
	10	+	-	-	+	+	4.4
	11	-	+	-	+	+	4.0
	C1*	C	+	0	-	-	0.0
	C4	C	+	0	+	+	0.0
1R†	R5	-	-	+	-	-	3.5
	R8	+	+	+	-	-	3.1
	R10	+	-	-	+	+	3.1
	R11	-	+	-	+	+	>5.0
	RC1	C	+	0	-	-	0.0
	RC4	C	+	0	+	+	0.0
2	1	-	-	-	-	+	4.3
	4	+	+	-	-	+	3.4
	14	+	-	+	+	-	4.1
	15	-	+	+	+	-	2.5
	C6	C	+	0	+	-	0.0
	C7	C	+	0	-	+	0.0
3	6	+	-	+	-	+	3.8
	7	-	+	+	-	+	4.0
	9	-	-	-	+	-	3.7
	12	+	+	-	+	-	>4.3
	C2	C	+	0	+	-	0.0
	C3	C	+	0	-	+	0.0
4	2	+	-	-	-	-	>4.4
	3	-	+	-	-	-	2.4
	13	-	-	+	+	+	3.8
	16	+	+	+	+	+	>4.4
	C5	C	+	0	-	-	0.0
	C8	C	+	0	+	+	0.0

> Exceeded detection limit; ± Factor levels, refer to Table 5.4(b);

†1R - Repeat of Block 1

*C1 - Control 1

Table 5.4(b) Screening Experiment: Factors and Levels

Factors	Levels (-)	Levels (+)
(A) Ozone dose (mg/L)	1.5	3.0
(B) Temperature (°C)	5	22
(C) Contact time (min)	2	7
(D) Turbidity (NTU)	0	5
(E) pH	5.7	7.6

Table 5.5(a) Specific Ozone Residual Experiment: Design and bench-scale ozone inactivation results for *G. muris* in ozone demand-free phosphate buffer.

Trial	Residual Ozone (mg/L)	Utilized Ozone (mg/L)	Factor				Inactivation, $\log \frac{N}{N_o}$
			A	B	C27	C12	
1	0.35	0.24	-	-	0		2.1
2	0.35	0.24	+	-	0		>5.1
3	0.40	0.33	-	+	0		4.5
4	0.36	0.46	+	+	0		>5.1
5	0.37	0.29	0	0	0		3.6
R5*	0.37	0.34	0	0	0		3.6
6	0.30	0.32	+	0	0		4.2
R6*	0.40	0.24	+	0	0		4.2
7	0.29	1.25	+	+	+		4.0
8	0.42	0.35	0	0	+		3.5
9	0.38	0.09	-	+	-	+	3.8
10	0.41	0.07	0	0	-	+	3.1
11	0.41	0.12	+	-	-	+	3.2
12	0.39	0.03	-	-		-	3.1
13	0.40	0.03	-	+		-	3.1
14	0.20	0.08	+	0		-†	2.2

> Exceeded detection limit; ± Factor levels, refer to Table 5.5(b);

*R5, R6, repeat of trial 5 and 6

†Contact time was 0.5 min

Table 5.5(b) Specific Ozone Residual Experiment: Factors and Levels

Factors	Levels		
	(-)	(0)	(+)
(A) Temperature (°C)	5	14	22
(B) pH	5.7	6.7	7.6
(C27) Time (min)	2	5	7
(C12) Time (min)	1	1.5	2

Table 5.6(a) Natural Water Experiment: Design and bench-scale *G. muris* ozone inactivation results.

Water (Date)	Trial	Residual Ozone (mg/L)	Utilized Ozone (mg/L)	Factor A	Factor B	Factor C25	Factor C410	Inactivation, $\log \frac{N}{N_o}$
NSR	1	0.25	0.71	-	-	-		2.7
(14-01-91)	2	0.70	1.88	+	+	-		3.7
	3	0.83	1.87	+	-	+		>5.0
	4	0.24	2.79	-	+	+		2.0
GVRD	5	0.65	1.45	+	-	+		4.1
(22-02-91)	6	0.15	0.94	-	-	-		2.1
	7	0.35	2.27	-	+	+		>5.1
	8	0.83	1.54	+	+	-		3.1
GVRD	9	0.37	0.91	-	-		-	3.1
(13-03-91)	10	0.76	0.90	+	-		-	3.1
	11	0.77	1.28	+	-		+	3.0
	12	0.34	1.37	-	-		+	3.3

> Exceeded detection limit; ± Factor levels, refer to Table 5.6(b);
NSR = North Saskatchewan River water;
GVRD = Greater Vancouver Regional District water.

Table 5.6(b) Natural Water Experiment: Factors and Levels

Factors	Levels (-)	Levels (+)
(A) Ozone residual (mg/L)	0.30	0.80
(B) Temperature (°C)	5	22
(C25) Time (min)	2	5
(C410) Time (min)	4	10

were positive, and inactivation is shown as being greater than the detection limit. Significant factors and interactions affecting inactivation were determined by ANOVA (Table 5.7). The error variance in Table 5.7 was estimated from the smallest interactions with the aid of a half-normal probability plot (Daniel, 1959). The error variance was 0.0746 with 7 d.f. In Table 5.7 the CD and CE interactions were not significant, indicating there were no batch-to-batch differences in cyst preparations.

The ANOVA results indicated that main effects applied ozone dose, temperature and pH were significant ($P \leq 0.05$). The two-factor interactions AE (ozone dose x pH) and BE (temperature x pH) and the main effect pH were significant ($P \leq 0.01$). Contact time was not a significant factor over the range examined (2–7 min) nor was it involved in any interactions that influenced the inactivation of *Giardia* cysts.

Temperature and pH are known to be two of the more important factors influencing ozone decomposition (Gurol and Singer, 1982; Bablon *et al.*, 1991a). On the basis of the screening experiment it was not possible to tell if the significance of temperature and pH was due to effects on ozone residual, or due to a direct influence on the *G. muris* cyst itself.

5.4.2.2 Specific ozone residual experiment

Inactivation results for the specific ozone residual experiment are shown in the last column of Table 5.5(a). The significant factors and interactions were determined by ANOVA for contact times less than or equal to 2 min [Table 5.8(a)] and greater than 2 min [Table 5.8(b)]. A pooled estimate of error variance was obtained from the mean square of the insignificant interactions in Tables 5.8(a) and 5.8(b) as determined by regression analysis and half-normal probability plots. Contact time for periods less than or equal to 2 min and temperature at all contact times were significant ($P \leq 0.05$). However, for contact times greater than 2 min, pH and all interactions including pH were also significant ($P \leq 0.05$). Apparent Ct products were calculated for each combination of ozone residual and contact time for each set of temperatures, and were plotted in Figure 5.11. Figure 5.12 presents the *G. muris* inactivations in terms of

Table 5.7 Screening Experiment ANOVA

Factor	MS	MS/MS$_d$†
(A) O3 Dose	0.423	5.66*
(B) Temperature	0.456	6.10*
(C) Time	0.141	1.88
(D) Turbidity	0.276	3.69
(E) pH	0.951	12.74**
AB	0.076	1.01
AC	0.031	0.41
AD	0.141	1.88
AE	1.626	21.78**
BC	0.010	0.13
BD	0.723	9.68*
BE	1.000	13.40**
CD	0.123	1.64
CE	0.160	2.14
DE	0.003	0.03

MS = mean square of the total effect

†MS$_d$ = 0.0746 with 7 degrees of freedom

$^*P \leq 0.05$; $^{**}P \leq 0.01$.

Table 5.8(a) ANOVA for a contact time less than or equal to two minutes in the specific ozone residual experiment

Factor	MS	MS/MS_d †
(A) Temperature	0.743	10.7*
(B) pH	0.004	0.1
(C) Time12	0.605	8.7*

MS = mean square of the total effect

†MS_d = 0.0696 with 6 degrees of freedom. Based on pooled estimate of error variance in laboratory water specific ozone residual experiment (Table 5.5).

*$P \le 0.05$.

Table 5.8(b) ANOVA for a contact time greater than two minutes in the specific ozone residual experiment

Factor	MS	MS/MS_d †
(A) Temperature	4.50	64.7**
(B) pH	2.88	41.4**
(C) Time27	0.32	4.6
AB	2.88	41.4**
AC	0.32	4.6
BC	0.50	7.2*
ABC	3.38	48.6**

MS = mean square of the total effect

†MS_d = 0.0696 with 6 degrees of freedom. Based on pooled estimate of error variance in laboratory water specific ozone residual experiment (Table 5.5).

*$P \le 0.05$; **$P \le 0.01$.

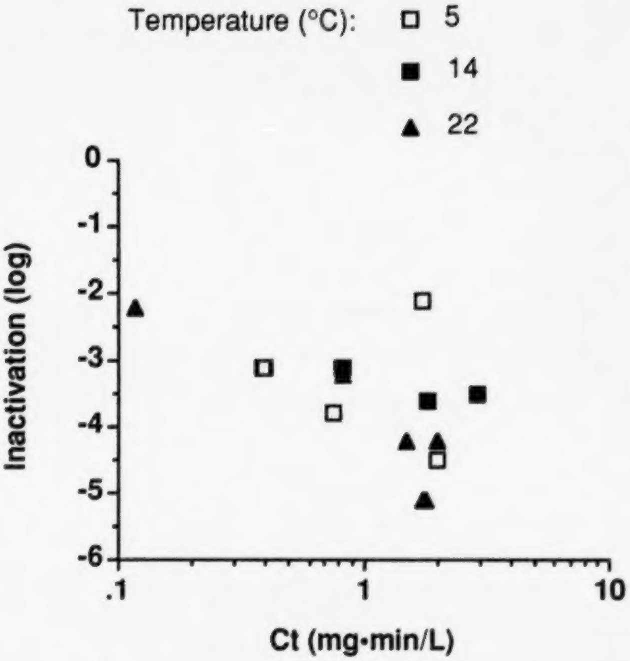


Figure 5.11 Effect of the apparent Ct on *G. muris* inactivation in specific ozone residual experiment.

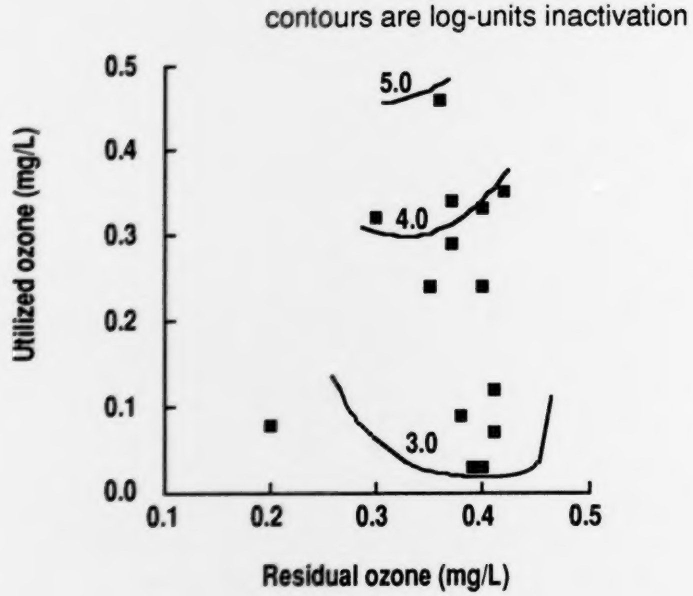


Figure 5.12 *Giardia muris* inactivation contours as a function of residual and utilized ozone in the specific ozone residual experiment.

utilized ozone and residual ozone (contours fitted quadratically). The effects of temperature and pH are accounted for by the utilized term which is directly affected by these factors. The sum of ozone residual and utilized ozone for each coordinate is the applied ozone dose. The inactivation data at about 0.4 mg/L of ozone residual in Figure 5.12 ranges from about 3 log inactivation for essentially zero ozone utilized (applied ozone equals residual ozone) to 5 log inactivation for utilized ozone of 0.45 mg/L (applied ozone is 0.45 + 0.40 or 0.85 mg/L). The higher inactivations occurred when the ozone utilized was more than ten times that for the lower inactivations.

5.4.2.3 Natural waters experiment

Inactivation results for the natural waters experiments are shown in the last column of Table 5.6(a). Inspection of Table 5.6(a) indicates that residual ozone was the most important factor and contact time the least important factor in the ozone inactivation of *G. muris* in NSR water. Unexpectedly, contact time between 2 and 5 min was found to be more significant than either temperature or residual ozone for *G. muris* inactivation in GVRD water, as can be seen from trials 5–8 in Table 5.6(a). However, contact time between 4 and 10 min was not significant, as indicated by the similar inactivations for trials 9–12 in Table 5.6(a). Apparent Ct products were calculated for each natural surface water for each set of temperatures and were plotted in Figure 5.13. Figure 5.14 presents the natural water inactivation response surface in terms of residual and utilized ozone (contours fitted quadratically).

5.4.3 Experimental Factors in Laboratory Water

In the screening and constant residual experiment it was observed that ozone rapidly inactivated *G. muris* and that contact time beyond 2 min resulted in little additional inactivation. The screening experiment demonstrated that applied ozone dose (and resulting residual) was significant ($P \leq 0.05$), with greater inactivation associated with the higher applied ozone dose. This is consistent with the ozone inactivation of other microorganisms where ozone concentration has been the most

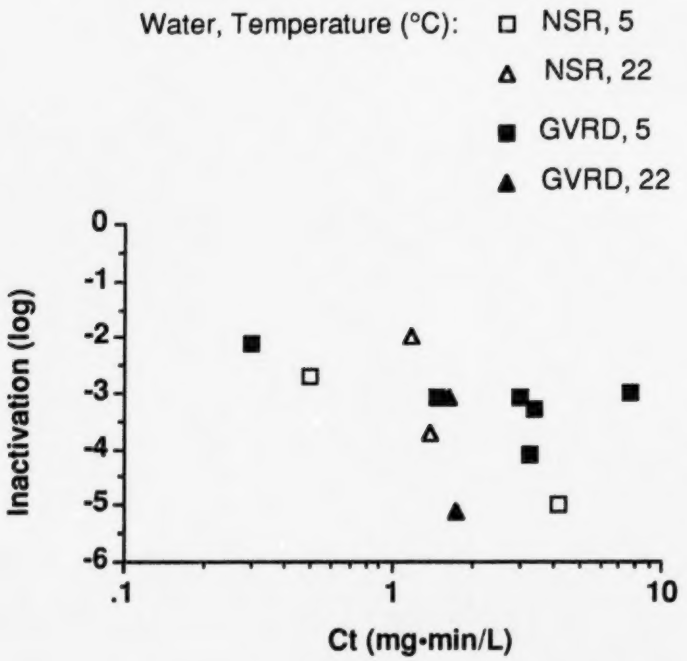


Figure 5.13 Effect of the apparent Ct on *G. muris* inactivation in North Saskatchewan River (NSR) water and Greater Vancouver Regional District (GVRD) water.

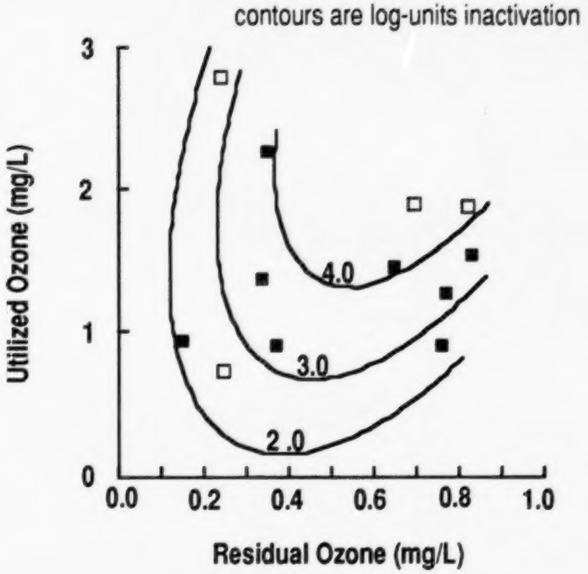


Figure 5.14 *Giardia muris* inactivation contours as a function of residual and utilized ozone for the natural waters experiment (open symbols, North Saskatchewan River water; solid symbols, Greater Vancouver Regional District water).

important design factor and ozone inactivation is relatively independent of contact time after an initial stage of about 60 s in clean water (Coin *et al.*, 1964; Farooq *et al.*, 1977; Finch *et al.*, 1988; Katzenelson *et al.*, 1974; Roy *et al.*, 1982).

pH was a significant factor ($P \leq 0.05$) in the screening experiment, which examined contact times from 2 to 7 min, as well as in the specific ozone residual experiment for contact times greater than 2 min. pH was not a significant factor in the specific ozone residual experiment for contact times less than or equal to 2 min. Other researchers have reported that pH had a minimal effect on ozone disinfection of water (Farooq *et al.*, 1977; Venosa, 1972).

Temperature was a significant factor ($P \leq 0.05$) in all experiments performed [Tables 5.7, 5.8(a) and 5.8(b), p. 122 and 123]. However, the sign of the total effect was not consistently positive or negative, indicating that the effect of increased temperature on *G. muris* cyst inactivation was adverse in some cases, and beneficial in others. One hypothesis which could account for the importance of temperature is that the *G. muris* organism itself is more susceptible to ozone inactivation at the higher temperatures. It is known that the viability of *G. muris* cysts is prolonged when the organism is stored at 5°C (deRegnier *et al.*, 1989; Wickramanayake *et al.*, 1985). Another factor is that chemical reaction rates double for each 10°C rise in temperature. Thus, at a temperature of 22°C, one could expect that the reaction of molecular ozone and *G. muris* cysts would be almost four times faster than at 5°C. Yet another factor is that cold temperatures increase ozone stability.

The turbidity caused by adding bentonite to the laboratory water did not have a significant effect on ozone inactivation; however, it was observed to have a beneficial effect on the physical removal of *Giardia* through microflocculation enhancement by the use of ozone. This phenomenon following the ozonation of turbid waters containing *Giardia* has been previously reported (Wallis *et al.*, 1990). Other researchers reported that turbidity adversely affected ozone inactivation of *E. coli*, human enteric viruses, porcine picornavirus and coliphage f_2

(Sproul *et al.*, 1979). However the sizes of these organisms are smaller than that of *Giardia*, and thus entrapment in suspended particles may not be as significant an effect with *Giardia* (Hoff, 1978).

Utilized ozone is computed as applied ozone less residual ozone, and combines the effects of contact time, temperature and pH. Parameters influencing ozone utilization in water include: pH, which influences ozone residual; temperature, which influences kinetic rate constants; the presence or absence of initiators or promotors, which influences ozone decomposition; and organic and inorganic impurities, which react selectively with ozone as defined by the competing reaction rate kinetics. The importance of utilized ozone in *G. muris* inactivation is demonstrated graphically in Figure 5.12 (p. 125) for the specific ozone residual data. In the screening experiment it was postulated that the significant interactions (ozone dose x pH and temperature x pH) also indicated the importance of utilized ozone. Trial 1 (the uppermost open square) in Figure 5.11 (p. 124) may be an outlier while trial 3 appears to be more appropriate relative to the other data. However, trial 1 was conducted at pH 5.7, and consequently had a utilized ozone value that was 0.73 times that of trial 3, which was conducted at pH 7.6.

In Figure 5.11 the variance in *G. muris* inactivation increases as the Ct increases. This may be due to the confounding of pH and temperature with time in achieving a target ozone residual of 0.4 mg/L at the end of the specified contact time. Longer contact times required more applied ozone at higher temperatures and pH than at lower temperatures and pH. Consequently, ozone doses fluctuated markedly accounting for the significant variation in the response of the *G. muris* cysts to ozone as Ct (or time) increased. The effects of pH are confused with time effects because pH also alters the rate of ozone decomposition. The Ct product in Figure 5.11 does not account for the decomposition of ozone, while the utilized ozone term in Figure 5.12 does.

5.4.4 Experimental Factors in Natural Waters

NSR water was hard and of high alkalinity and pH while GVRD water was extremely soft and low in alkalinity and pH (Table 4.1, p. 68).

There was a much higher level of organic matter, turbidity and absorbance in the GVRD water than in the NSR water. Thus parameters which affected ozone decomposition and consumption were significantly different for these two waters. Increased alkalinity inhibits the decomposition process though possibly to a limited extent given the relatively low concentrations found even in hard waters. The effect of pH was very apparent in these two waters and NSR water decomposed more readily than GVRD water. The higher organic carbon content of the GVRD water would be expected to result in more competing ozone reactions than in the NSR water. This is illustrated by the values of w (equation [2.33], p. 43) and Ω_M (Section 2.3.3) in these waters. The specific ozone utilization rate, w, is 11.1 h^{-1} for NSR water (13-02-91 sample), resulting in a 5 min ozone utilization of 0.93 mg/L. For GVRD water (22-02-91 sample) w is 18.2 h^{-1}, however the water quality parameters for GVRD water are outside the range for which the regression coefficients in equation [2.33] were determined. Ω_M can be estimated using a value of 0.25 mg/L per mg/L DOC, resulting in 0.4 mg/L for NSR water (13-02-91 sample) and 2.3 mg/L for GVRD water (22-02-91 sample). It is not surprising that higher ozone doses were required in the GVRD water to achieve the same inactivations as in the NSR water.

In all natural water trials except one, a higher value of utilized ozone resulted in greater inactivation. In NSR water (pH 8.3) utilized ozone correlated directly with residual ozone, and the inactivation values (mean ± SD) were 2.4 ± 0.5 log-units at an ozone residual of approximately 0.3 mg/L and 4.4 ± 0.9 log-units at the 0.8 mg/L residual. In GVRD water (pH 6.3) utilized ozone appeared to correlate with contact time rather than residual ozone.

Figure 5.14 indicated that *G. muris* inactivation in the natural waters was dependent on residual and utilized ozone as was the case in the laboratory waters. When residual ozone was less than or equal to 0.3 mg/L, N/N_0 was less than 3 log inactivation regardless of the value of utilized ozone. Figure 5.14 also indicated that for residual ozone values greater than 0.4 mg/L, more inactivation occurred in NSR water than in GVRD water. Several factors likely contributed to this: the higher utilized ozone levels in the NSR water, and the greater organic levels and concomitant competing reactions in the GVRD water. Contact time beyond 2 min resulted in additional inactivation in GVRD water, but not in NSR water. This may also be an effect of the higher organic levels in GVRD water. It has been shown using bacteria and wastewater that the presence of suspended particles required substantially longer ozone contact times to achieve inactivations equivalent to those obtained when suspended matter was absent (Kirk *et al.*, 1972).

Figure 5.13 (p. 127) presents data indicating that it was more difficult to achieve 2 or 3 log inactivation of *G. muris* cysts in the natural waters at 22°C than at 5°C. Temperature has opposing influences on ozone stability and disinfection efficiency. It should be noted that 4 log inactivation of *G. muris* occurs more readily at 22°C than at 5°C. While it has been observed by other researchers that at higher temperatures the increased reaction rate effect exceeds that of ozone instability (Farooq *et al.*, 1977; Roy *et al.*, 1982), this was not the case for *G. muris* cyst inactivation in the natural waters used in this study, although it appeared to be so in the laboratory water. This can be explained by the increased contact time required for inactivation of *G. muris*, particularly in natural waters, compared with that required for other organisms which can often be inactivated in less than 60 s. It appears that the positive effect of colder temperatures in preserving ozone residual exceeds negative kinetic influences in the ozone inactivation of *G. muris* cysts.

5.5 COMPARISON OF *G. MURIS* AND *G. LAMBLIA*

5.5.1 Objective

The objective of this experiment was to compare *G. muris* and *G. lamblia* infectivity following ozonation using the C3H/HeN mouse-*G. muris* and Mongolian gerbil-*G. lamblia* models. A semi-quantitative determination of *G. lamblia* inactivation was made. The bench-scale disinfection procedure and phosphate buffer were used.

5.5.2 Discussion

Experimental conditions are summarized in Table 5.9(a) and inactivation results for *G. lamblia* and *G. muris* are presented in Table 5.9(b). Further details on experimental conditions and animal responses for the *G. lamblia* trials can be found in Appendix F (Table F.3, exp. 43 data). *G. muris* inactivation results from other experimental trials made under similar ozonation conditions were used for comparison. For example, trial 30-13 in Table 5.9(a) designates trial 13 of experiment 30. Details on these *G. muris* trials can also be found in Appendix F. From the data available it was not possible to choose three *G. muris* trials to match the *G. lamblia* trials precisely; however, applied ozone doses and apparent Ct values are reasonably close. All trials including control trials had sodium formate added to neutralize ozone. The amount used, 50 µL of 1 M solution, was not toxic to either *G. lamblia* or *G. muris* cysts. All animals in control trials were positive in both the gerbil and mouse models.

The semiquantitative *G. lamblia* cyst inactivation determination method is presented in Table 5.10. In Table 5.10, N was the total number of viable cysts in the experimental cohort, estimated using a literature value for the number of *G. lamblia* cysts that would cause infection to a given proportion of Mongolian gerbils (Figure 1 in Schaefer *et al.*, 1991). For consistency, the N_0 definition used for the mouse model was also used for the gerbil model. This was defining N_0 as the total inoculum in the control cohort, determined by four replicate hemocytometer counts.

G. muris cysts were typically collected on day 7 and cyst preparations were of high quality as shown in Figure 5.6 (p. 104). *G. lamblia* cysts were collected from day 7 to day 14 and cyst preparations required more effort in purification. Fluorogenic staining of the *G. lamblia* cyst preparation resulted in: 15 percent FDA-positive, 4 percent EB-positive and 81 percent unstained. It is not known if the unstained population is viable or nonviable. If the *G. lamblia* cyst preparation was just 50 percent viable, the difference in inactivation estimates would be only 0.3 log-units less than if the preparation was 100 percent viable.

Table 5.9(a) Experimental conditions for *G. muris* and *G. lamblia* trials in ozone demand-free phosphate buffer (pH 6.8; 22°C).

Trial	Contact Time (min)	Residual Ozone (mg/L)	Utilized Ozone (mg/L)	Applied Ozone (mg/L)	Apparent Ct (mg•min/L)
G. lamblia					
1	5	0.8	0.6	1.4	4.0
2	5	0.5	0.4	0.9	2.5
3	2	0.2	0.3	0.5	0.4
C	5	-	-	-	-
G. muris					
30-13	7	0.6	1.0	1.6	4.2
33-A2	5	0.4	0.2	0.6	2.0
41-6	2	0.4	0.1	0.5	0.8

C - control.

Table 5.9(b) Bench-scale ozone inactivations as estimated by the C3H/HeN mouse-*G. muris* and Mongolian gerbil-*G. lamblia* models.

Trial	Animals in Cohort	Positive Animals in Cohort	Inactivation, $\log \frac{N}{N_0}$
G. lamblia			
1	5	0 on day 14	≥3.8
2	5	0 on day 14	≥3.8
3	4	1 on day 14	3.0
C	5	5 on day 11	0.0
G. muris			
30-13	5	3 on day 8*	3.8
33-A2	5	4 on day 8	4.2
41-6	5	4 on day 6	3.2

*Three of 5 mice were positive for cysts on day 8; all 5 mice had trophozoites detected on day 8;

C - control.

Table 5.10 Semiquantitative determination of *G. lamblia* inactivation

Trial	Percent of Mongolian gerbils infected in experimental cohort			
	0%	25%	50%	75%
G. lamblia inoculum (cysts/gerbil)[†]	11	60	282	1,320
Viable cysts in experimental cohort, N				
1	≤55	-	-	-
2	≤55	-	-	-
3	-	240	-	-
Total number of cysts in control cohort, N_o				
C*	3.1×10^5	3.1×10^5	3.1×10^5	3.1×10^5
Inactivation, $\log \frac{N}{N_o}$				
1	≥3.8	-	-	-
2	≥3.8	-	-	-
3	-	3.0	-	-

[†] From Figure 1 of Schaeffer *et al.*, 1991;

* Value is 2.5×10^5 for trial 3 because there were only 4 gerbils.

The gerbil and mouse models are very different, as discussed in Sections 2.1.5, 4.3.7 and 4.3.8. The mouse model uses the latent period to provide a quantitative estimate of inactivation while the gerbil model provides a semiquantitative inactivation estimate. Therefore, this is not a strict mathematical comparison. On the basis of number of animals infected, trial 1 does not provide any additional information over trial 2. In trial 2, 0 out of 5 gerbils became infected, while 4 out of 5 mice became infected by day 8. On this basis, it appears that *G. lamblia* is less resistant to ozone than *G. muris*. However, it should be noted that the *G. muris* inocula were about twice as large as those for *G. lamblia*, mainly due to the greater difficulty in obtaining *G. lamblia* cysts. In trial 3 the contact time and ozone residual were decreased in an effort to have at least part of the gerbil cohort become positive. Under these conditions 1 out of 4 gerbils became infected whereas 4 out of 5 mice became infected on day 6. The *G. muris* inactivation in trial 3 was 3.2 log-units compared with a *G. lamblia* inactivation of 3.0. The result of trial 3 indicates that *G. lamblia* cysts may be of similar resistance to ozone as *G. muris* cysts at 22°C. Although the apparent Ct values in trial 3 are not the same, the applied ozone doses are. Additional observations and replicates to determine assay variability are required to make firm conclusions. A large amount of the uncertainty in *G. lamblia* inactivation estimates can be explained by the inherent variability of the biological assay and differences in host susceptibility. The *G. lamblia* ID_{50} as determined by probit analysis was reported to be 282 cysts; however, the 95 percent confidence interval for the ID_{50} estimate was 60 to 1,096 cysts (Schaeffer *et al.*, 1991).

In summary, the data reported here corroborates that of Wallis *et al.* (1990) who noted that there was little difference between *G. muris* and *G. lamblia* Ct values for three log-units inactivation under similar ozonation conditions for temperatures ranging from 6.7 to 9.0°C. In contrast Wickramanayake *et al.* (1985) reported *G. muris* to be 1.5 times more resistant to ozone than *G. lamblia* at 25°C on the basis of calculated Ct_{99} values. Further research is warranted.

5.6 PILOT-SCALE RESULTS

5.6.1 Objective

The objective of this experiment was to evaluate bench-scale results at pilot-scale using NSR water because:

1. Scale-up effects can seriously affect the performance of full-scale facilities, making bench-scale information questionable for use in design.
2. Kinetic models for full-scale design can be limited by the actual detention time in reactors. Larger-scale evaluation is especially important if bench-scale data are to be used for regulatory purposes.

The pilot-scale disinfection procedure was used with NSR water seeded with *G. muris* cysts. HPC bacteria indigenous to the test water were enumerated using R2A agar. Infectivity was determined using the C3H/HeN mouse-*G. muris* model.

5.6.2 Discussion

Experimental conditions and *G. muris* inactivation results are presented in Table 5.11. Further details on experimental conditions and animal responses for the trials can be found in Appendix F (Table F.4, exp. 39A and 39B for bench-scale; Table F.4, exp. 40 and 42 for pilot-scale). HPC bacteria inactivation results are given in Table F.5 in Appendix F.

Figure 5.15 shows *G. muris* and R2A HPC bacteria inactivation results at bench- and pilot-scales. Observations from Figure 5.15 include:

1. There is no apparent scale-up effect in the *G. muris* data. Most of the bench- and pilot-scale results agree within 0.5 log. The largest difference was between pilot-scale trials B and 2, performed on 14-11-90 and 31-01-91, respectively. The 2 log-unit lower *G. muris* inactivation in trial B can be attributed to its poorer water quality. The trial B sample had twice the turbidity and 4.3 times the TOC of the trial 2 sample.

Table 5.11 North Saskatchewan River Water: Pilot- and bench-scale *G. muris* ozone inactivation results.

Date	Trial	Scale	Residual Ozone (mg/L)	Utilized Ozone (mg/L)	Contact Time (min)	Inactivation, $\log \frac{N}{N_o}$
14-01-91	1	1	0.25	0.71	2	2.7
	2	1	0.70	1.88	2	3.7
	3	1	0.83	1.87	5	>5.0
	4	1	0.24	2.79	5	2.0
13-02-91	1	1	0.16	0.65	2	3.7
	2	1	0.67	1.63	5	4.6
	3	1	0.30	1.41	5	>5.2
	4	1	0.69	0.98	2	3.2
14-11-90	A	2	0.70	0.98	6.3	3.1
	B	2	0.38	0.51	3.2	1.6
31-01-91	1	2	0.21	1.26	4	2.7
	2	2	0.35	2.09	6	3.6
	3	2	0.80	2.87	10	>5.0
	4	2	1.23	3.72	13.5	>5.0

> Exceeded detection limit;
Scale - 1, bench-scale; 2, pilot-scale.

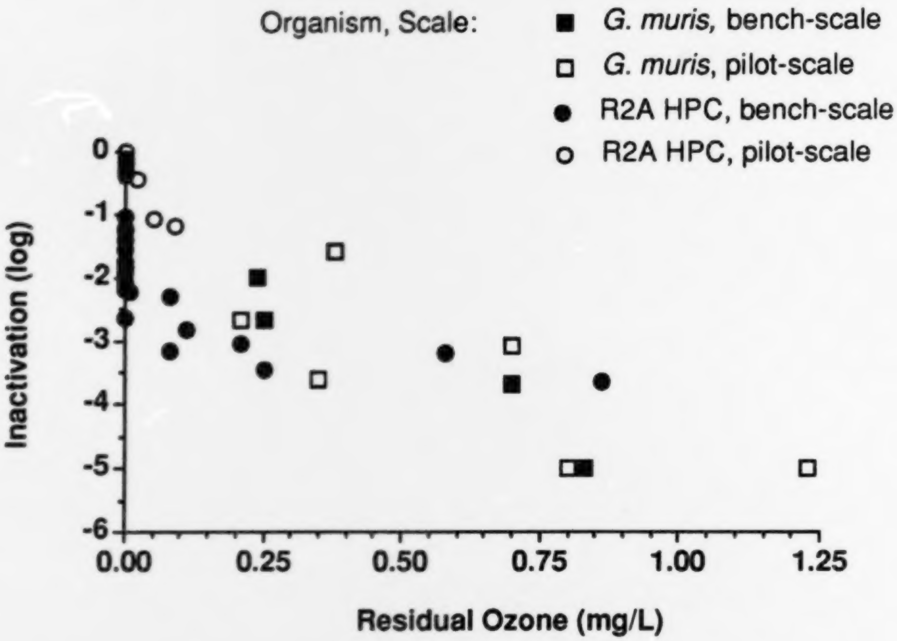


Figure 5.15 Inactivation of *G. muris* and heterotrophic plate count bacteria as a function of ozone residual at bench-scale and pilot-scale in North Saskatchewan River water.

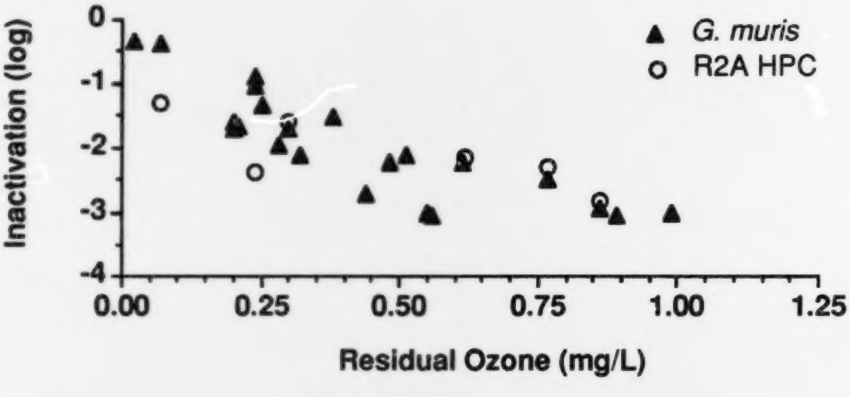


Figure 5.16 Inactivation of *G. muris* and heterotrophic plate count bacteria as a function of ozone residual at pilot-scale in Colorado River and State Project waters (R. Wolfe, Metropolitan Water District of Southern California, Los Angeles, Calif., 1990, Personal communication).

2. At first, it appears as though there may be a difference between the pilot- and bench-scale R2A HPC bacteria inactivation results. However, by interpreting the results in conjunction with the water quality characteristics of the samples (Table 4.1 and Table F.5, Appendix F), it can be seen that the pilot-scale sample was taken on 31-01-91 (pristine winter water quality) while the bench-scale data was sampled 22-04-91 (poor spring runoff water quality).
3. There is general agreement between the inactivation patterns of *G. muris* and R2A HPC bacteria.
4. Some of the variability in the inactivation results may be due to inactivation being functionally dependent on more parameters than just ozone residual.

The pilot-scale inactivation data shown in Figure 5.16 is for contact times of 3, 6 and 9 min and two surface waters (R. Wolfe, Metropolitan Water District of Southern California, Los Angeles, Calif., 1990, Personal communication). A number of additional observations can be made from Figures 5.15 and 5.16:

1. Agreement between *G. muris* and R2A HPC bacteria inactivation patterns is evident in Figures 5.16. Analysis by the MWDSC indicated that there was agreement between the inactivation of R2A HPC bacteria and *G. muris* up to 2.5 log-units (Scott *et al.*, 1992).
2. Three log-units of *G. muris* inactivation occurred at an ozone residual of 0.50 mg/L in Figure 5.15 (bench-scale data; pilot-scale trials A and B do not meet SWTR turbidity requirement), and approximately 0.80 mg/L in Figure 5.16. The biggest difference between the two studies was that infectivity was used in this study while the MWDSC used excystation. Another difference was that the current study used a Rushton turbine semi-batch, stirred-tank reactor for pilot-scale work while a countercurrent flow reactor was used by the MWDSC.

Pilot-scale trials were performed at 22°C. The four bench-scale trials at 1°C (exp. 39B data) were not included in Figure 5.15 because these results appeared highly variable and were the only trials performed at such a low temperature. A difference between the bench- and pilot-scale protocols was that ozone residual decreased at bench-scale but increased at pilot-scale. It should be noted that the dynamic forces which govern gas bubble formation in a reactor cannot be scaled. Therefore bubble size and bubble rise-rate will be the same at all scales. Because bench-scale reactors do not have the same mass-transfer characteristics as pilot- and full-scale reactors, bubbling ozone through a reactor at bench-scale will actually be a poorer simulation of full-scale performance than side-stream injection in a batch reactor (Finch and Smith, 1989).

5.7 ENGINEERING SIGNIFICANCE OF RESULTS

5.7.1 Process Design Protocol

Figure 5.17 uses quadratically fitted contours to show *G. muris* inactivation as a function of ozone residual and contact time for the viability comparison, specific ozone residual and natural water data sets. The response surface is not linear (i.e. it is not a family of straight lines) and cannot be adequately modelled by the Chick-Watson model. When ozone residual was less than 0.5 mg/L and contact time was less than 5 min, inactivation was less than 3 log. However, once the contact time was 5 min or more, and residuals were at least 0.5 mg/L, more than 3 log of *G. muris* cyst inactivation was consistently obtained. This "envelope" would yield an apparent Ct of 2.5 mg min/L, and is largely defined by the natural waters in the combined data set.

The U.S. disinfection regulations are given in terms of Ct and temperature (USEPA, 1991). The Ct system assumes that the pseudo first-order rate constant, k, follows the van't Hoff–Arrhenius relationship and that the disinfectant concentration is constant during the course of an experiment. The data reported here does not appear to support such a simple kinetic model largely due to the complexity of competing ozone

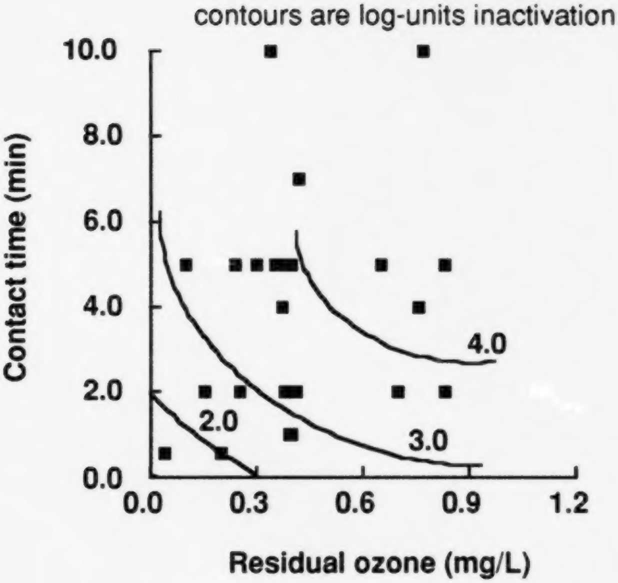


Figure 5.17 *Giardia muris* inactivation contours as a function of contact time and residual ozone in specific ozone residual, viability comparison and natural waters experiments.

reactions in natural water. Furthermore, the extrapolation of design criteria based on the Chick-Watson model beyond observed experimental conditions can result in gross errors (Hoff, 1987). The U.S. National Research Council (1980) originally adopted the Ct concept for comparing biocidal efficiency in their review of the disinfection literature; however, they cautioned that comparisons be made at the 99 percent inactivation level to minimize problems associated with initial lags and "tailing off". Other researchers have proposed alternate statistical techniques because Ct does not adequately describe nonidealities or the complete mathematics of kinetic expressions (Haas, 1987; Severin and Churn, 1987).

Even though the Chick-Watson model was found to be inadequate, kinetic data from selected natural water and laboratory water observations were used to produce the Watson plots shown in Figures 5.18 and 5.19 for comparative purposes only. Data for all temperatures (5–22°C) were pooled due to the limited number of observations available. The coefficient of dilution, n, is shown on the plots. In the Chick-Watson model n is an empirical factor, and n being greater than one suggests that residual ozone concentration may be more important than contact time, while n being less than one suggests that contact time may be more important. The n values were less than one in all cases except for 3 log inactivation in laboratory water, suggesting that contact time was more important than residual ozone concentration. The results of the screening experiment indicated that the opposite was true. The reason for the apparent contradiction is the assumption that the kinetics were pseudo first-order in nature. Wickramanayake *et al.* (1984b) reported n values of 1.1–1.2 for 3 log ozone inactivation of *G. muris* cysts in laboratory water.

Ct values from Figures 5.18 and 5.19 along with regulatory values for comparison (USEPA, 1991) are summarized in Table 5.12. Although the regulatory values contain a safety factor of two, they were just adequate for 3 log inactivation of *G. muris* cysts at 5°C for the natural waters used in this study, and inadequate for 3 log inactivation at 22°C. Contrary to the U.S. regulations (USEPA, 1991), it was found to be more

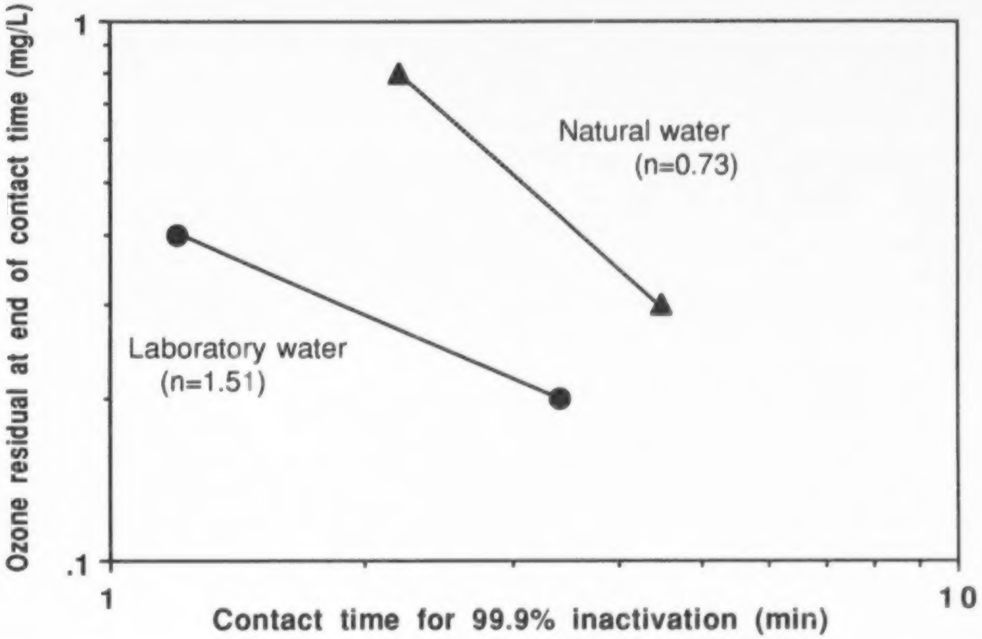


Figure 5.18 Watson plot of selected data from laboratory and natural water experiments for 99.9 percent inactivation of *G. muris* cysts.

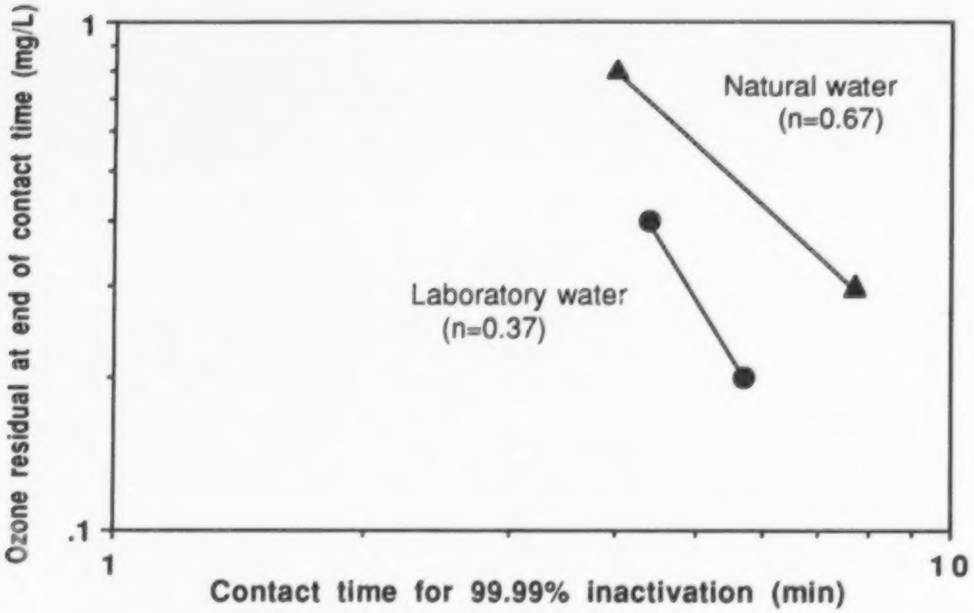


Figure 5.19 Watson plot of selected data from laboratory and natural water experiments for 99.99 percent inactivation of *G. muris* cysts.

Table 5.12 Experimental Ct values for inactivation of *G. muris* cysts by ozone (pH 5.7 to 8.3) and regulatory Ct values for *G. lamblia* cyst inactivation by ozone (pH 6 to 9)

Water type	Inactivation $\log\frac{N}{N_o}$	Estimated Ct values			
		This study[†]	EPA[*] 5°C	This study[†]	EPA[*] 22°C
Laboratory	3	0.30	1.9	0.30	0.62
	4	3.1	2.6[††]	3.1	1.22[††]
Natural	3	1.9	1.9	1.9	0.62
	4	3.4	2.6[††]	3.4	1.22[††]

[*] Table O-2, *Guidance Manual* (USEPA, 1991)

[†] This study pooled temperature data

[††] Extrapolation from Table O-2 (USEPA, 1991)

difficult to achieve 3 log inactivation of *G. muris* cysts in the natural waters at 22°C than at 5°C (Figure 5.13, p. 127). Further extrapolation of the recommended Ct values to 4 log inactivation results in theoretical Ct values which are 1.2–2.8 times too low when compared with the values determined here. Table 5.12 also indicates that it is approximately six times more difficult to achieve 3 log inactivation of *G. muris* cysts in the natural waters used in this study than in the laboratory water. This result supports the observation that carefully prepared microorganisms in demand-free buffers may underestimate the chemical dose necessary to achieve disinfection in natural waters (Berg, 1987).

It is suggested that the Ct concept may be inappropriate for regulating the design of ozone disinfection systems for unfiltered water supplies. An alternative empirical approach would be to use the 3 log contour "envelope" in Figure 5.17 (p. 142) to make predesign decisions concerning primary ozone disinfection systems. Figure 5.20 is a three dimensional portrayal of the data in Figure 5.17. Trials which had ozone residuals greater than or equal to 0.5 mg/L and contact times of at least 5 min had greater than 3 log-units of *G. muris* cyst inactivation (shaded area). A response surface was not fitted in Figure 5.20 to let the "data speak for itself". However, in the region $0.5 \leq C_r \leq 1.0$ mg/L and $t \leq 5$ min there is a plateau in the data. This is illustrative of some of the difficulties with kinetic modelling of ozone disinfection. The plateau would have a tailing appearance in a two dimensional plot of inactivation versus ozone residual.

The results of this work indicate that increased contact time cannot compensate for inadequacies in ozone dose in the ozone inactivation of *Giardia*. Contact time beyond 2 min was found to be unimportant in the laboratory water and also in the NSR water. In the GVRD water, which had twice the turbidity and nine times the DOC as the NSR water, contact time beyond 5 min was unimportant. Competing reactions caused by the greater amount of organic matter in the GVRD water could account for the greater contact time necessary to achieve *G. muris* inactivation in that water. However, contact times greater than 5 min do not appear to be necessary in the ozone inactivation of *G. muris*

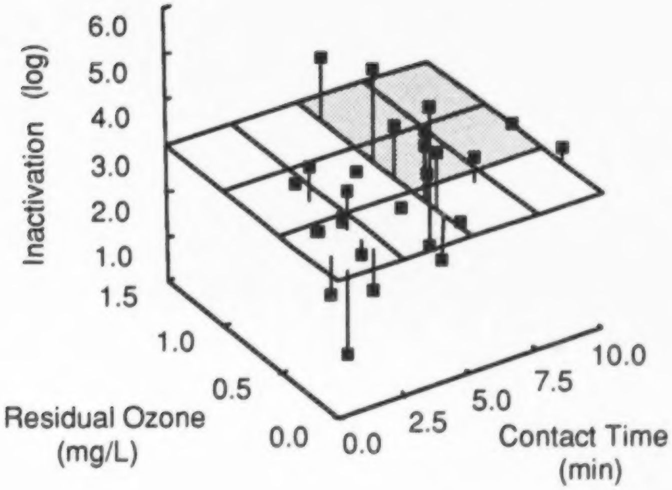


Figure 5.20 *Giardia muris* inactivation relative to 3 log-units as a function of contact time and residual ozone in specific ozone residual, viability comparison and natural waters experiments. Shaded area exceeds the empirical process design protocol.

in waters meeting the SWTR turbidity and coliform requirements. The understanding of interactions between temperature, pH, ozone dose and ozone decomposition kinetics is essential for the successful design of an ozone system for inactivation of *Giardia* cysts.

5.7.2 Ozone Inactivation Modelling and Analysis

Modelling is an iterative, circular process (Box *et al.*, 1978). The primary objective of this section was to consolidate previous results and use modelling as a tool to gain further insight into the relative importance of residual and utilized ozone when inactivating *Giardia* cysts. The numerical prediction of high levels of *G. muris* cyst inactivation was of secondary importance. Detailed variance analysis, joint confidence regions of parameters, and sum of squares plots, or equivalent, were beyond the scope of this section.

5.7.2.1 Data selection

The data used for modelling was developed by considering all C3H/HeN mouse-*G. muris* model results except the screening experiment (Tween 20 not used, see Section 4.4.1) so that the data set would be as large as possible; however, criteria based on experimental findings were applied so that the data would be homogeneous. The first criterion was that only laboratory and NSR water results with contact times of 2 min or more, and GVRD water results with contact times of at least 5 min were included. This eliminated the need for kinetic considerations in the analysis but still retained the majority of the data. Figure 5.21 illustrates the lack of time dependence beyond 2 min in the ozone inactivation of *G. muris* cysts in laboratory water.

The second criterion for inclusion in the data set for modelling was that temperature be in the range of 5–22°C. The four bench-scale trials at 1°C were excluded because these results appeared erratic, temperature significantly affected the ozone inactivation of *G. muris* cysts ($P \leq 0.05$) and temperature may affect the *G. muris* organism itself (Wickramanayake *et al.*, 1985).

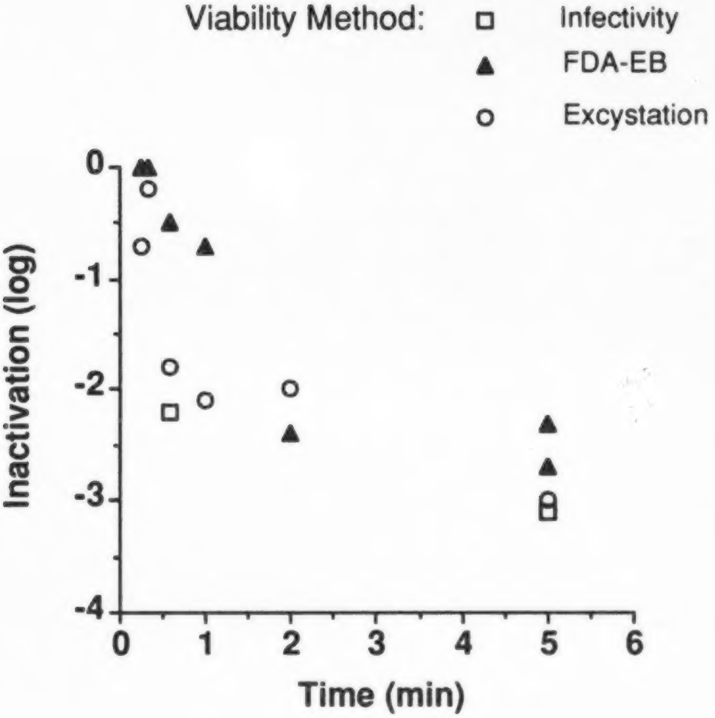


Figure 5.21 *Giardia muris* cyst ozone inactivation as a function of contact time in viability comparison experiment (ozone residual 0.1–0.2 mg/L; pH 6.7; 22°C). Viability determined by C3H/HeN mouse infectivity, fluorescein diacetate-ethidium bromide (FDA-EB) staining and *in vitro* excystation.

More ozone was utilized in natural waters than in laboratory waters. Utilized ozone values were 0.1 and 0.3 mg/L, respectively, for 3 and 4 log-units inactivation in the specific ozone residual experiment (Figure 5.12, p. 125); whereas in the natural waters these inactivations were observed when utilized ozone was 0.7 and 1.5 mg/L (Figure 5.14, p. 128). The higher utilized ozone in the natural waters was due to ozone demand. Table 4.1 indicated that ozone demand ranged from about 0.5 mg/L for the GVRD water to 1.0 mg/L for NSR water at 5°C. Figures 5.22 and 5.23 summarize the relationship between applied ozone dose, ozone residual and ozone demand in the natural waters. Ozone demand was calculated as the sum of immediate demand and the nonlinear portion of 5 min decomposition (Section 4.1.2). Ozone decomposition is a physical property of aqueous ozone and laboratory water first-order ozone decomposition plots were typically linear (Sections 2.3.2 and 5.1.1). Thus, ozone demand is considered a property of a natural water matrix and would be zero in a properly prepared laboratory water. This is somewhat different than the definition used in the recently published proposed standard method, which includes all decomposition as part of ozone demand and does not specify a standard temperature or contact time for the test (APHA *et al.*, 1991). It was hypothesized that if the ozone demand was subtracted from utilized ozone values in natural waters, *G. muris* cyst inactivation results from laboratory and natural waters could be pooled for modelling purposes.

Figure 5.24 presents *G. muris* inactivation as a function of ozone utilized per cyst in selected experiments (refer to Table I.1 in Appendix I). The objectives in preparing Figure 5.24 were to calculate an overall ozone demand and to determine if some early experiments (exps. 18 and 24, Table H.5, Appendix H) conducted using deionized water as the suspending medium could be pooled with the rest of the data. Using Figure 5.24, the ozone demand in the natural water can be calculated as $[(0.100 - 0.015)10^{-9}\text{g/cyst}] \times [2 \times 10^{6}\,\text{cyst/0.2L}]$ or 0.85 mg/L. This is a reasonable average value for the NSR and GVRD waters (Table 4.1, p.68). Although the deionized water data appeared comparable with the phosphate buffer data (Figure 5.24), it was not included in the

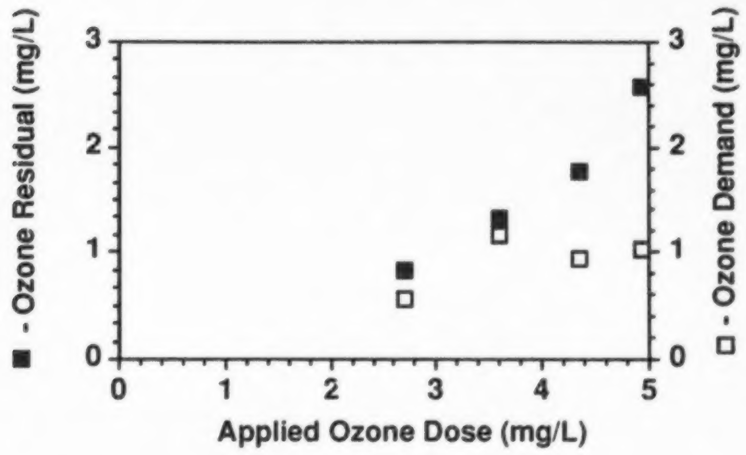


Figure 5.22 Relationship between applied ozone dose, ozone residual and ozone demand in North Saskatchewan River water for a contact time of 5 min (5°C; pH 8.3).

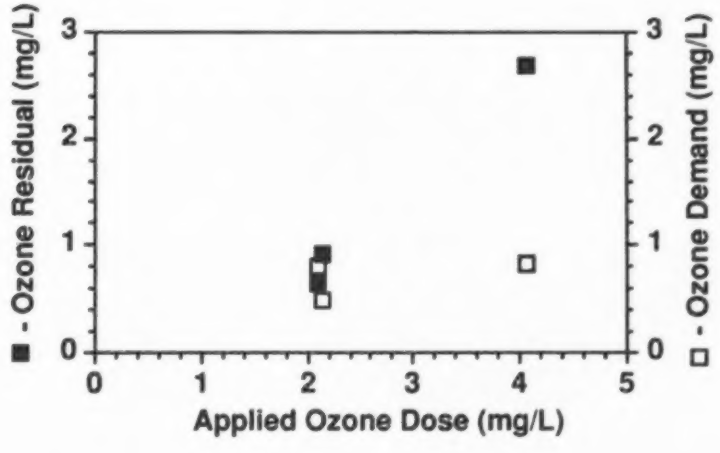


Figure 5.23 Relationship between applied ozone dose, ozone residual and ozone demand in Greater Vancouver Regional District water for a contact time of 5 min (5°C; pH 6.3).

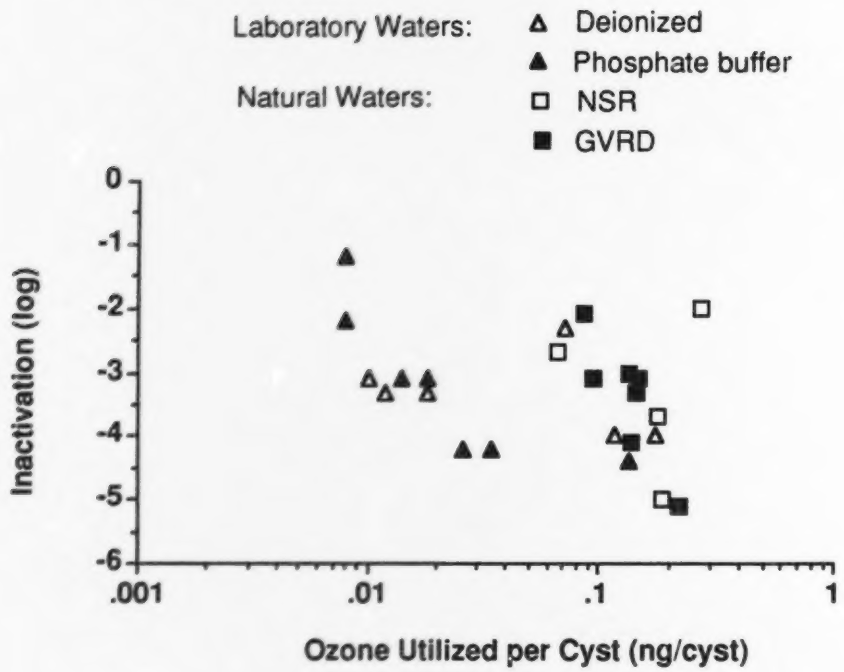


Figure 5.24 Effect of ozone utilized per cyst on *G. muris* inactivation in laboratory, North Saskatchewan River (NSR) and Greater Vancouver Regional District (GVRD) waters.

data set for modelling because there were too many differences in experimental protocol (refer to Sections 4.1.1, 4.3.8.1 and 4.4.1) and the lack of ionic strength in deionized water was suspected to cause very high *G. muris* cyst lysing. The open outlying square in Figure 5.24 is from a trial that had a large ozone demand relative to the other NSR water trials (trial 39A-4, Table J.1, Appendix J).

The results from the comparison of ozone and ozone–hydrogen peroxide experiment were not included in the data set for modelling because they were conducted in laboratory buffer systems engineered to completely utilize ozone or preserve applied ozone almost entirely. In addition, the purpose of modelling was to confirm the ozone and ozone–hydrogen peroxide experiment conclusion independently. The importance of residual ozone in disinfection is assumed to be through the direct action of the ozone molecule while utilized ozone acts through ozone decomposition products such as the hydroxyl radical.

The pooling of pilot- and bench-scale results was supported by the lack of scale-up effects as indicated in Figure 5.15. An ozone demand of 1.20 mg/L in exp. 40 was estimated from the TOC and turbidity values of the sample relative to a NSR sample taken two weeks earlier which had the ozone demand determined. Exp. 42 pilot-scale results were not used because the turbidity and TOC values were higher than for other samples and it was not possible to accurately predict the ozone demand.

Combining all results gave a data set with 29 cases, 15 from laboratory waters and 14 from natural waters (Table 5.13). The data consists of 18 trials at low pH (pH 5.7, 6.3 or 6.7) and 11 trials at high pH (pH 7.6 or 8.3). Of the 29 trials, 10 were at 5°C, 4 at 14 or 15°C, and 15 at 22°C. The temperature and pH subsets were too small to permit modelling. Plots of *G. muris* cyst inactivation versus residual ozone, utilized ozone less ozone demand and average ozone are shown in Figures 5.25, 5.26 and 5.27. There is less variation in the ozone residual plot (Figure 5.25) than in the utilized ozone less ozone demand plot (Figure 5.26). A single trial in Figure 5.26 appears to be an outlier; however, the trial had a very small ozone residual and a large utilized

Table 5.13 Summary of *G. muris* cyst inactivation data for modelling.

Trial	Residual Ozone C_r (mg/L)	Utilized Ozone C_u (mg/L)	Ozone Demand C_d (mg/L)	Average Ozone C_{av}# (mg/L)	DOC (mg/L)	Inactivation (log N/N_o)
33-A1	0.30	0.32	-	0.44	0.09	4.2
33-A2	0.40	0.24	-	0.50	0.09	4.2
33-B1	0.10	0.16	-	0.16	0.09	3.1
33-B2	0.10	0.20	-	0.18	0.09	3.1
33-C1	1.30	1.25	-	1.90	0.09	4.4
36-1	0.35	0.24	-	0.45	0.09	2.1
36-2	0.35	0.24	-	0.45	0.09	>5.1
36-3	0.40	0.33	-	0.54	0.09	4.5
36-4	0.36	0.46	-	0.57	0.09	>5.1
36-5	0.37	0.29	-	0.49	0.09	3.6
36-6	0.37	0.34	-	0.52	0.09	3.6
41-3	0.38	0.09	-	0.40	0.09	3.8
41-4	0.41	0.07	-	0.42	0.09	3.1
41-5	0.42	0.35	-	0.57	0.09	3.5
41-6	0.41	0.12	-	0.45	0.09	3.2
39A-1	0.25	0.71	0.32	0.45	0.99	2.7
39A-2	0.70	1.88	0.89	1.20	0.99	3.7
39A-3	0.83	1.87	0.55	1.49	0.99	>5.0
39A-4	0.24	2.79	1.54	0.87	0.99	2.0
39C-1	0.65	1.45	0.80	0.98	9.15	4.1
39C-3	0.35	2.27	1.37	0.80	9.15	>5.1
39D-1	0.37	0.91	0.37	0.64	9.15	3.1
39D-2	0.76	0.90	0.30	1.06	9.15	3.1
39D-3	0.77	1.28	0.49	1.17	9.15	3.0
39D-4	0.34	1.37	0.50	0.78	9.15	3.3
40-1	0.21	1.26	1.20*	0.24	1.62	2.7
40-2	0.35	2.09	1.20*	0.80	1.62	3.6
40-3	0.80	2.87	1.20*	1.64	1.62	>5.0
40-4	1.23	3.72	1.20*	2.49	1.62	>5.0

# C_{av}, $C_{av} = C_r + 0.5 \times (C_u - C_d)$

*, estimate;

>, detection limit of C3H/HeN mouse model.

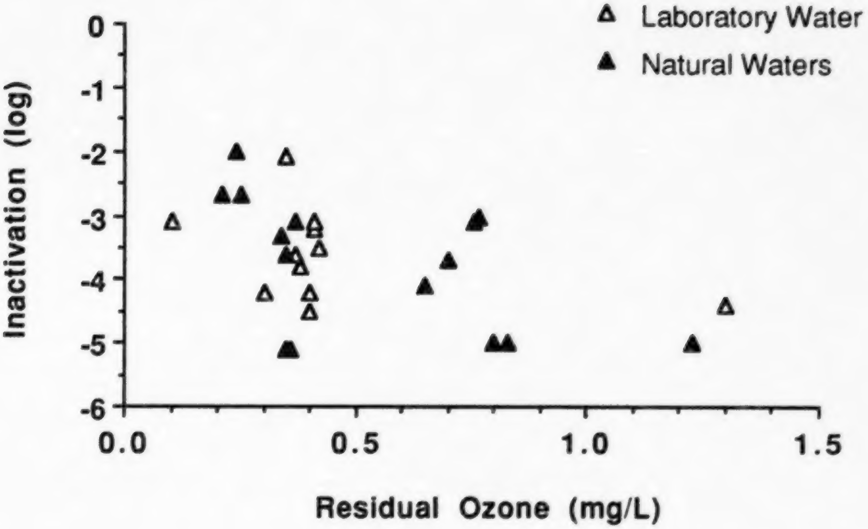


Figure 5.25 Effect of residual ozone and water type on *G. muris* inactivation in the modelling data set

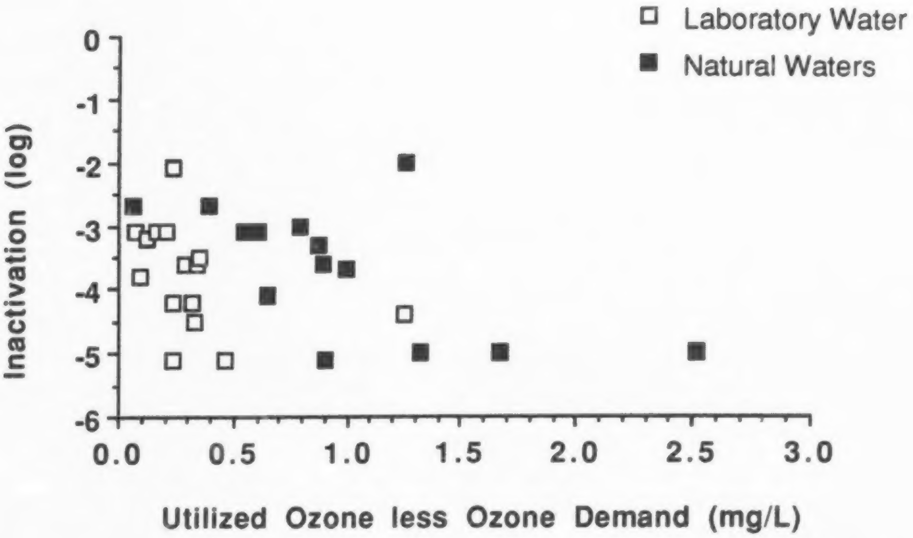


Figure 5.26 Effect of utilized ozone less ozone demand and water type on *G. muris* inactivation in the modelling data set

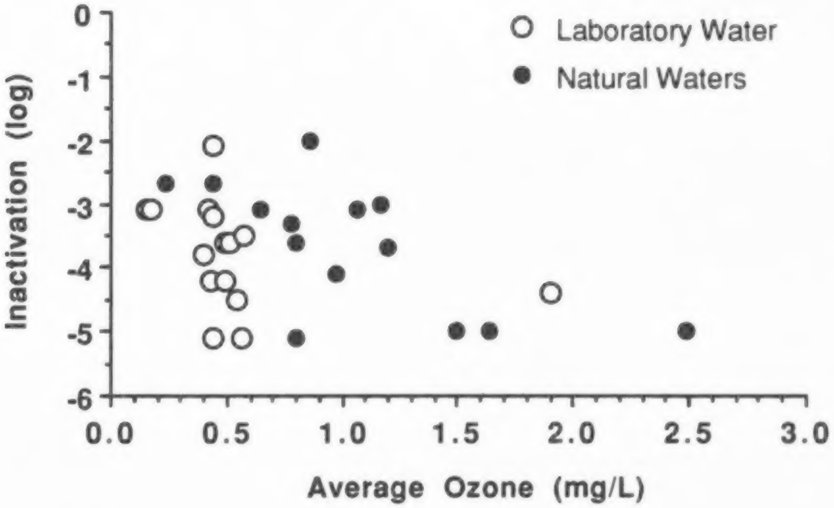


Figure 5.27 Effect of average ozone and water type on *G. muris* inactivation in the modelling data set

ozone value (trial 39A-4, Table 5.13). Figure 5.28 shows *G. muris* inactivation contours as a function of residual ozone and utilized ozone less ozone demand for this data. Figure 5.28 indicates that the data set for modelling has a cluster of data with an ozone residual of approximately 0.4 mg/L.

5.7.2.2 Model formulation

The model was identified on the basis of:

residual ozone = f(applied ozone, pH) [5.3]

utilized ozone = f(applied ozone, competing reactions, temperature) [5.4]

$$\log \frac{N}{N_o} = \text{f(residual ozone, utilized ozone)} \qquad [5.5]$$

Thus the independent variables included temperature and pH effects. Contact time also influences residual and utilized ozone, however data was selected to eliminate the need for kinetic considerations (Section 5.7.2.1). The form of model used was:

$$Y = f(X_1 + X_2 + X_1X_2) \qquad [5.6]$$

where Y is log cyst survival, X_1 is a residual ozone term, X_2 is an utilized ozone term and X_1X_2 is an interaction term. The initial model was:

$$\log \frac{N}{N_o} = - [\theta_1 C_r^{\theta_2} + \theta_3 (C_u - C_d)^{\theta_4} + \{\theta_1 C_r^{\theta_2}\} \times \{\theta_3 (C_u - C_d)^{\theta_4}\}] \qquad [5.7]$$

where: N_o	is the initial concentration of microorganisms;
N	is the concentration of surviving microorganisms when C_u ozone is utilized;
C_r	is the ozone residual in mg/L;
C_u	is utilized ozone in mg/L;
C_d	is the ozone demand in mg/L; and
θ_1, θ_2, θ_3 and θ_4	are nonlinear model parameters.

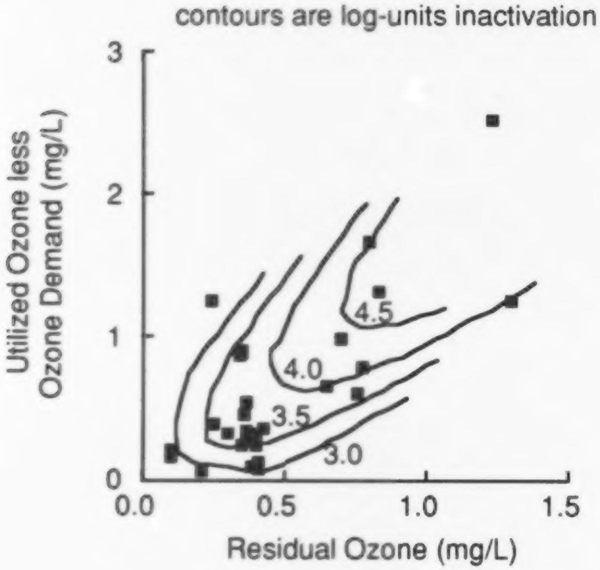


Figure 5.28 *Giardia muris* inactivation contours as a function of residual ozone and utilized ozone less ozone demand in the modelling data set.

Equation [5.7] is very general and includes a coefficient and an exponent for each independent variable. Utilized ozone less ozone demand was used for the second independent variable to permit the pooling of laboratory and natural water results (Section 5.7.2.1).

The next step in model building is parameter estimation. In 6 of the 29 trials in the data set, no mice became positive and inactivation was greater than the detection limit (Table 5.13, p. 155). One of the poorer approaches to model parameter estimation would be to simply delete this data (Helsel, 1990). However, because squaring a large value increases its impact, the use of ordinary least squares for parameter estimation with censored data is prohibited (Helsel, 1990). Instead, the selected loss function used the least absolute values of deviations in observed dependent variables from their estimates (Wilkinson and Leland, 1989):

$$\text{Minimize} \quad | y - y^* | \qquad [5.8]$$

where: y is the dependent variable; and

y^* is the model-predicted dependent variable.

Other researchers have reported relative least squares to be more suitable than ordinary least squares in some environmental engineering applications (Saez and Rittmann, 1992).

Model parameters and diagnostics including the confidence limits of parameters, asymptotic correlation matrix of parameters and residual plots are provided in Appendix K. Table 5.14 lists observed and estimated inactivations. Diagnostic analysis was generally satisfactory except that the 95 percent confidence interval of θ_2 included zero (Appendix K.1). However, at this point it was realized that the variances of utilized ozone less ozone demand in the laboratory and natural waters may not be homogeneous (Figure 5.26, p. 157). An *F*-test confirmed that (C_u - C_d) variances in the laboratory and natural waters were not homogeneous. The variances of these two waters appear equivalent in Figure 5.24 (p. 153); however logarithmic plots can be deceptive.

Table 5.14 Observed and estimated *G. muris* cyst inactivation by ozone

Trial	Inactivation (log N/N_O)	Model equation number [5.7]		Model equation number [5.9]	
		Estimate	Residual	Estimate	Residual
	y	y^*	ε	y^*	ε
33-A1	4.2	3.52	0.68	3.69	0.51
33-A2	4.2	3.49	0.71	3.70	0.50
33-B1	3.1	3.02	0.08	3.10	0.00
33-B2	3.1	3.09	0.01	3.17	-0.07
33-C1	4.4	4.58	-0.18	4.92	-0.52
36-1	2.1	3.45	-1.35	3.65	-1.55
36-2	>5.1	3.45	>1.65	3.65	>1.45
36-3	4.5	3.61	0.89	3.81	0.69
36-4	>5.1	3.71	>1.39	3.90	>1.20
36-5	3.6	3.54	0.06	3.74	-0.14
36-6	3.6	3.60	0.00	3.80	-0.20
41-3	3.8	3.15	0.65	3.35	0.45
41-4	3.1	3.10	0.00	3.30	-0.20
41-5	3.5	3.64	-0.14	3.86	-0.36
41-6	3.2	3.26	-0.06	3.47	-0.27
39A-1	2.7	3.55	-0.85	3.29	-0.59
39A-2	3.7	4.26	-0.56	4.00	-0.30
39A-3	>5.0	4.47	>0.53	4.18	>0.82
39A-4	2.0	4.06	-2.06	3.67	-1.67
39C-1	4.1	4.03	0.07	3.44	0.66
39C-3	>5.1	4.01	>1.09	3.32	>1.78
39D-1	3.1	3.79	-0.69	3.19	-0.09
39D-2	3.1	4.04	-0.94	3.47	-0.37
39D-3	3.0	4.18	-1.18	3.56	-0.56
39D-4	3.3	3.98	-0.68	3.30	0.00
40-1	2.7	2.91	-0.21	2.68	0.02
40-2	3.6	4.00	-0.40	3.60	0.00
40-3	>5.0	4.59	>0.41	4.16	>0.84
40-4	>5.0	4.99	>0.01	4.53	>0.47
Mean		3.76		3.64	
SD		0.52		0.45	

y, observed dependent variable; SD, standard deviation;

y^*, dependent variable predicted from nonlinear model;

>, detection limit of C3H/HeN mouse model.

The ozone demand calculation method used in this work is affected by pH. This is the reason that although GVRD water had nine times the DOC as the NSR water, its ozone demand was lower than that of the NSR water. A transformation was desired which would compensate for this deficiency as well as stabilize variances. The selected transformation was $(C_u - C_d)/\sqrt{DOC}$. Ozone metameters are frequently normalized by the DOC or TOC values of natural waters (Chang and Singer, 1991). The square root transformation is relatively mild (Box *et al.*, 1978), and in this application improves the applicability of the model to both laboratory and natural waters. The factor $1/\sqrt{DOC}$ is a surrogate for competing reactions not included in C_d. Variances of $(C_u - C_d)/\sqrt{DOC}$ in the laboratory and natural waters are homogeneous (Table J.1, Appendix J). The revised model was:

$$\log \frac{N}{N_o} = - [\theta_1 C_r^{\theta_2} + \theta_3 (\frac{C_u - C_d}{\sqrt{DOC}})^{\theta_4} + \{\theta_1 C_r^{\theta_2}\} \times \{\theta_3 (\frac{C_u - C_d}{\sqrt{DOC}})^{\theta_4}\}] \qquad [5.9]$$

where: DOC is dissolved organic carbon in mg/L, and the other parameters are as defined for equation [5.7].

Model parameters and diagnostics for equation [5.9] are provided in Appendix K.2. There was no obvious lack-of-fit of the data to the model. The high negative correlation between θ_1 and θ_3 is a reflection of the fact that residual ozone and utilized ozone are often negatively correlated. A challenge in the data being modelled is that trials 36-1 and 36-2 had identical C_r and C_u values, yet N/N_o values were 2.1 and >5.1 log-units, respectively. There would be questions regarding the validity of the data except that trial 36-1 was conducted at 5°C and trial 36-2 was conducted at 22°C. At the same time, a weakness of the model is that temperature is not explicitly included.

The ratio of the first to second terms in equations [5.7] and [5.9] gives a general idea of the relative importance of residual and utilized ozone when inactivating *Giardia* cysts. A t-test was used to confirm if the two terms were statistically different. A summary of the contribution of each term in the models is presented in Table 5.15. Note

Table 5.15 Summary of *G. muris* cyst inactivation by ozone in the modelling data set as predicted by two models

Model equation number	Final value of loss function†	Mean value of model term number			Mean model-predicted inactivation	Ratio:
		1	2	3	y*	Term 1 / Term 2
[5.7]	17.540	1.81#	0.69	1.26	3.76	2.62
[5.9]	16.252	1.27#	1.04	1.33	3.64	1.22

† Loss function used was least absolute values;
# Statistically significant difference from term 2 value, $P \leq 0.001$;
y^*, dependent variable predicted from nonlinear model.

that equation [5.9] is a preferable model compared with equation [5.7] because the final value of the loss function is 16.252, more than a full unit lower than previously.

5.7.2.3 Discussion

The data presented in Table 5.15 indicates that ozone residual appears to be more important than utilized ozone when inactivating *G. muris* cysts. In both models the C_r term was greater than the $(C_u - C_d)$ or $(C_u - C_d)/\sqrt{DOC}$ terms, and the C_r term accounted for a significantly larger amount of model-predicted inactivation ($P \leq 0.001$). This supports the conclusion of the ozone and ozone–hydrogen peroxide experiment.

The sensitivity of the dependent variable in equation [5.9] to various values of C_r, C_u, C_d and DOC is presented in Table 5.16. Figure 5.29 is a plot of the simulated inactivations. Note the general similarity between the observed data in Figure 5.20 and the simulated data in Figure 5.29. The major difference is that the range of the simulated data in not as large. Conditions in Figure 5.29 which satisfy the SWTR *Giardia* requirement are ozone residuals greater than or equal to 0.5 mg/L and a $(C_u - C_d)/\sqrt{DOC}$ value of at least 0.10.

A difficulty with models is that they are sometimes very specific to the data for which they are developed. Data from Table 4.13 of Metropolitan Water District of Southern California and James M. Montgomery Consulting Engineers (1991) was used to compare model-predicted and observed *G. muris* cyst ozone inactivations. These data were for State Project Water tests with a temperature range of 13–21°C and TOC values of 2.4–2.6 mg/L. DOC was estimated to be 2.5 mg/L and C_d was estimated as 0.63 mg/L. Model-predicted inactivation was 2.74 log-units (simulation 100, Table 5.16) compared with an observed inactivation of 2.03 log-units (mean of 10 tests) for C_r of 0.3 mg/L (mean). The predicted inactivation was within the range of the observations. Differences may be attributable to imprecision of C_d estimate, excystation used by MWDSC while equation [5.9] is based on infectivity, and t_{10} values of 3.9 min in the MWDSC tests, although HDT was 12 min.

Table 5.16 Sensitivity analysis of *G. muris* cyst inactivation by ozone as predicted by equation [5.9]

Simu-lation	Independent Variables				Transformed Variable	Dependent Variable Estimate
	C_r (mg/L)	C_u (mg/L)	C_d (mg/L)	DOC (mg/L)	$\frac{C_u - C_d}{\sqrt{DOC}}$	y^*
100	0.3	0.7	0.63	2.5	0.04	2.74
101	0.2	1.3	0.5	2.5	0.51	3.30
102	0.2	1.3	0.5	10.0	0.25	3.09
103	0.2	1.3	1.0	2.5	0.19	3.01
104	0.2	1.3	1.0	10.0	0.09	2.83
105	0.2	1.3	1.2	2.5	0.06	2.73
106	0.2	1.3	1.2	10.0	0.03	2.58
107	0.3	1.2	0.5	2.5	0.44	3.39
108	0.3	1.2	0.5	10.0	0.22	3.18
109	0.3	1.2	1.0	2.5	0.13	3.02
110	0.3	1.2	1.0	10.0	0.06	2.85
111	0.4	1.1	0.5	2.5	0.38	3.44
112	0.4	1.1	0.5	10.0	0.19	3.23
113	0.4	1.1	1.0	2.5	0.06	2.94
114	0.4	1.1	1.0	10.0	0.03	2.77
115	0.5	1.0	0.5	2.5	0.32	3.46
116	0.5	1.0	0.5	10.0	0.16	3.25
117	0.5	1.0	0.9	2.5	0.06	3.01
118	0.5	1.0	0.9	10.0	0.03	2.84
119	0.5	2.0	0.5	2.5	0.95	3.85
120	0.5	2.0	0.5	10.0	0.47	3.60
121	0.5	2.0	1.0	2.5	0.63	3.70
122	0.5	2.0	1.0	10.0	0.32	3.46
123	0.5	2.0	1.8	2.5	0.13	3.19
124	0.5	2.0	1.8	10.0	0.06	3.01
125	0.8	1.7	0.5	2.5	0.76	3.95
126	0.8	1.7	0.5	10.0	0.38	3.70
127	0.8	1.7	1.0	2.5	0.44	3.75
128	0.8	1.7	1.0	10.0	0.22	3.52
129	0.8	1.7	1.6	2.5	0.06	3.16
130	0.8	1.7	1.6	10.0	0.03	2.99

C_r, residual ozone; C_u, utilized ozone; C_d, ozone demand;
DOC, dissolved organic carbon;
y^*, log-units inactivation predicted by model equation [5.9].

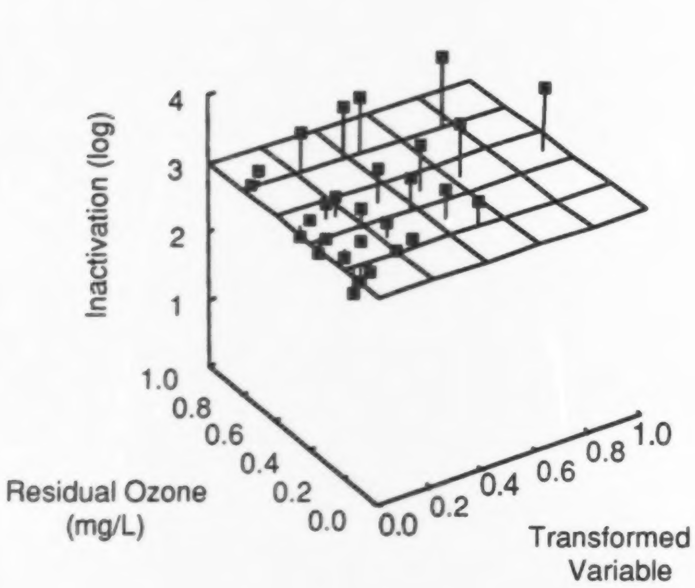


Figure 5.29 Simulated *G. muris* inactivation relative to 3 log-units as a function of residual ozone and a transformed variable that depends on utilized ozone, ozone demand and dissolved organic carbon $[(C_u - C_d)/\sqrt{DOC}]$.

Equation [5.9] was developed as an inferential statistical tool and it may be overparameterized for general prediction of *G. muris* cyst ozone inactivation. Equation [5.9] should not be used for design. Additional modelling work is required to develop design equations which should be validated using independent data. Further analysis may indicate that the ozone demand subtracted in equations [5.7] and [5.9] may in fact account for some inactivation. Until more work is done, the process design "envelope" developed in Section 5.7.1 can be used to achieve the objective of inactivating 3 log-units of *Giardia*.

5.7.3 Integration of Proposed Protocol with *Guidance Manual*

Appendix O in the "Guidance Manual for Compliance with the Filtration and Disinfection Requirements for Public Water Systems Using Surface Water Sources" provides recommendations on the definitions of C and t in ozone disinfection (USEPA, 1991).

The completely mixed bench-scale and semi-batch stirred tank pilot-scale reactors used in this work were theoretically perfect contactors. Each particle in the water was exposed to the same concentration of ozone for the same period of time. To compensate for nonidealities such as short-circuiting in full-scale systems, the t of 5 min in this work should be interpreted as the t_{10} requirement of flow-through reactors in the *Guidance Manual*.

The definition of ozone concentration is complicated because of ozone decomposition and various possible flow regimes depending on contactor configuration (Section 2.5.3). Ozone contactor configurations favored for primary disinfection in water treatment are multi-chamber bubble diffusers because plug-flow conditions are increased minimizing contactor volumes and short-circuiting (IOA, 1990). The bench-scale ozonation protocol used herein may conceptually be regarded as a very short CSTR stage during ozone addition followed by a reactive stage for the specified contact time. The ozone concentration C reported in this work was generally C_r, the ozone residual at the end of the specified contact time. C_r, or C_{out} as it is designated in Appendix O of the *Guidance Manual* has the advantages of being readily observable and indicating a surplus level of ozone over competing ozone reactions.

The use of average ozone for the characteristic concentration C is also discussed in Appendix O of the *Guidance Manual*. Various definitions have been used for average ozone. The MWDSC in their pilot-scale work reported average ozone as [(applied ozone dose + ozone residual)/2] (Wolfe *et al.*, 1989b). To exclude the effect of ozone demand, the definition of average ozone used in this work was (Table 5.13, p.155):

$$C_{av} = C_r + 0.5 \times (C_u - C_d) \qquad [5.10]$$

or, $$C_{av} = C_r + 0.5 \times [(C_o - C_r) - C_d] \qquad [5.11]$$

where: C_{av} is the calculated average ozone value in mg/L;

C_r is the ozone residual in mg/L;

C_u is utilized ozone in mg/L;

C_d is the ozone demand in mg/L; and

C_o is the applied ozone dose in mg/L.

Equation [5.11] reduces to $[C_{av} = (C_o + C_r)/2]$ when C_d is zero.

Average ozone was found to be less suitable than other ozone metameters in bench-scale work performed here. Difficulties include:

1. there is a loss of information by averaging (i.e. Figure 5.27, p. 158 has less information than Figures 5.25 and 5.26, p. 156 and p. 157);
2. weighting ozone dose and residual ozone equally (C_{in} and C_{out} as defined in *Guidance Manual*; C_o and C_r as defined in this document) may not be justified because C_r appears to be more important in disinfection;

3. comparing average ozone values is not meaningful unless waters are of similar temperature, pH and are ozonated for similar contact times; and
4. high ozone demand waters could have high average ozone values, but little inactivation (i.e. trial 39A-4, Table 5.13, p. 155).

5.7.4 Microflocculation Effects of Ozone

In the screening experiment three ozonated trials with turbidity artificially introduced had cyst recovery that was an order of magnitude larger than in the controls. This phenomenon was presumed to be due to the effects of microflocculation. In these trials inactivation was estimated by using the actual ozonated trial inoculum size for the denominator term in equation [4.8].

Microflocculation is the flocculation of soluble micropollutants from water which can then be removed by filtration (Rice, 1989). Polar groups such as the carboxyl and hydroxyl groups are capable of hydrogen bonding in the presence of polyvalent metal cations and yield complexed higher molecular weight materials that are insoluble (Rice *et al.*, 1981).

A related phenomenon is the complex effect ozone has on particle stability in natural waters. It has been shown that for optimal ozone-induced coagulation, the hardness to TOC ratio should be greater than 25 mg hardness as calcium carbonate per mg of carbon with ozone doses of about 0.4 to 0.8 mg per mg carbon (Chang and Singer, 1991). These ratios for the two surface waters used were: hardness to TOC ratio of 145 and ozone to TOC ratio of 0.8-2.5 for NSR water, and hardness to TOC ratio of 0.5 and ozone to TOC ratio of 0.1-0.2 for GVRD water. Consequently, significant ozone-induced coagulation could be expected for the NSR water.

Hoff and Akin (1986) have reported that particulates associated with cell debris and wastewater effluent solids markedly decreased virus and bacteria inactivation rates by chlorine, chlorine dioxide and ozone.

When viruses and bacteria were adsorbed on clays, inactivation rates were affected only minimally. A comprehensive study on the effect of particulates on ozone disinfection included bacteria and viruses, but not *Giardia* (Sproul *et al.*, 1979).

Clearly, when ozone is added to natural waters there are many interacting variables. Pilot-scale experiments using the natural waters to be treated under a variety of seasonal conditions should be conducted prior to the design of full-scale facilities.

5.7.5 Public Health Protection

Although this work shows that ozone alone can achieve 3 log-units of *G. muris* cyst inactivation, operational criteria of actual contact time (HDT and t_{10}), ozone residual, applied ozone dose and influent water quality would have to be closely monitored to ensure successful operation. Should any part of the ozonation system fail, or short-circuiting occur in the contactor, public health could be compromised. A multi-barrier approach of chemical pretreatment prior to granular media filtration would have less inherent risk.

6. CONCLUSIONS

Ozone disinfection of water containing *Giardia* is complicated due to problems in determining *Giardia* viability, the definition of ozone concentration, and ultimately, in a gas-liquid reactor, defining the contact time. The use of the C3H/HeN mouse-*G. muris* model appears to be an effective means for determining the appropriate ozonation conditions for preventing transmission of infective *Giardia* cysts. The labor intensive nature of the C3H/HeN mouse-*G. muris* model currently restricts its use to researchers; however, the results have practical application. The conclusions of this work are:

1. The mouse model is highly sensitive and can detect greater than 5 log-units inactivation of *G. muris* cysts. In addition to being capable of detecting high level inactivation, the C3H/HeN mouse-*G. muris* model is capable of detecting inactivation as low as 1.2 log-units.

2. There were no significant differences ($P \leq 0.05$) between infectivity and *in vitro* excystation, or between fluorogenic staining and excystation for up to 99.9 percent inactivation of *G. muris* cysts. Fluorogenic staining significantly overestimated viability compared with infectivity ($P \leq 0.05$).

3. The C3H/HeN mouse-*G. muris* model was capable of measuring 1.5 to 2 log inactivation higher than *in vitro* excystation or fluorogenic staining. Should *Giardia* inactivation requirements be increased, infectivity may have to be used exclusively due to its high sensitivity.

4. The phosphate-bicarbonate buffer system, which had negligible decomposition of ozone, had significantly greater ($P \leq 0.05$) inactivation of *G. muris* cysts than that observed in the phosphate buffer system with peroxide where the ozone was completely decomposed in less than 60 s. By inference, the direct action of the ozone molecule was the most significant factor for inactivation of *G. muris*.

5. Ozone alone is a better disinfectant than ozone–hydrogen peroxide.

6. Temperature, pH, applied ozone dose and ozone dose x pH and temperature x pH interactions had statistically significant ($P \leq 0.05$) effects on *G. muris* cyst infectivity.

7. It may be more difficult to achieve 3 log inactivation of *G. muris* cysts at 22°C than at 5°C in certain waters. Higher temperatures increase the dissipation of ozone while colder temperatures tend to preserve ozone residual.

8. Contact time had more effect in some waters than in others. Contact time in demand-free 0.05 M phosphate buffer and North Saskatchewan River water was not significant beyond two min ($P \leq 0.05$). Contact times greater than five min were not significant in Greater Vancouver Regional District water.

9. The Ct concept, a simplification of the Chick-Watson model, should be reexamined for the purposes of predicting ozone inactivation of *G. muris* cysts. The data presented here indicates that the ozone inactivation of *G. muris* cysts is very complex and is not adequately predicted by Ct.

10. The use of a design "envelope" such as a minimum contact time of 5 min and a minimum ozone residual of 0.5 mg/L is recommended as a means of achieving the Surface Water Treatment Rule objective of inactivating 3 log-units of *Giardia*. The 5 min contact time should be interpreted as the t_{10} requirement of flow-through reactors for integration with values published in the *Guidance Manual*.

11. Some of the Ct values published in the *Guidance Manual* for ozone inactivation of *Giardia* may be too small when applied to natural waters.

12. There were no apparent differences between the bench- and pilot-scale *G. muris* cyst inactivation results.

13. Nonlinear modelling indicated that ozone inactivation of *G. muris* cysts is a function of residual and utilized ozone. The utilized ozone value used in the model was transformed to $(C_u - C_d)/\sqrt{DOC}$ to account for competing reactions in waters with ozone demands up to approximately 1 mg/L and DOC values up to 10 mg/L. pH and temperature effects were implicitly included by their influence on residual and utilized ozone levels. The residual ozone term accounted for a significantly larger amount of model-predicted inactivation ($P \leq 0.001$) than the utilized ozone term. This is additional inferential evidence of the importance of the direct action of the ozone molecule in disinfection. The model also indicates that the Surface Water Treatment Rule *Giardia* requirement is met if the ozone residual is at least 0.5 mg/L and the value of $(C_u - C_d)/\sqrt{DOC}$ is at least 0.10.

7. RECOMMENDATIONS

Recommendations for future work include:

1. Additional work should be done to expand the infectivity data set, particularly at pilot-scale using more natural surface waters. Sufficient replicates should be made to permit calculation of the 95 percent confidence limits of inactivation estimates.
2. At colder temperatures (5°C and less) results were more variable. Work should be done to try to ascertain why this is so. In addition, a set of inactivation data at 1°C should be developed.
3. A study should be made on the mode of action of ozone on *Giardia* cysts. This understanding would permit inactivation data to be applied more generally rather than being species-specific and would assist in the development of chemical kinetic models.
4. Further work should be done on *Giardia* cyst inactivation kinetics, the results of which could be used to optimize ozone reactor design. The maintenance of optimized ozone residual levels would decrease operating costs, and the design of contactors for minimum contact times would reduce capital costs.
5. A well designed comparison between the susceptibilities of *G. muris* and *G. lamblia* cysts to ozone in both laboratory and natural waters is required. There may be less difference between the susceptibilities of *G. muris* and *G. lamblia* cysts to ozone than has been previously thought and further investigation is warranted.
6. Additional microbial indicator studies should be done for *G. lamblia*. Heterotrophic plate count bacteria might be useful.

8. REFERENCES

Abbaszadegan, M., Gerba, C.P. and Rose, J.B. 1991. Detection of *Giardia* cysts with a cDNA probe and applications to water samples. Applied and Environmental Microbiology **57**:927-931.

Akin, E.W. and Jakubowski, W. 1986. Drinking water transmission of giardiasis in the United States. Water Science and Technology **18**(10):219-226.

Alberta Environment. 1988. Standards and guidelines for municipal water supply, wastewater and stormwater drainage facilities. April, 1988.

APHA, AWWA, WPCF. 1989. Standard methods for the examination of water and wastewater. American Public Health Association, 17th edition, Washington, DC.

APHA, AWWA, WPCF. 1991. Standard methods for the examination of water and wastewater. American Public Health Association, Supplement to the 17th edition, Washington, DC.

Bablon, G., Bellamy, W.D., Billen, G., Bourbigot, M., Daniel, F.B., Erb, F., Gomella, C., Gordon, G., Hartemann, P., Joret, J., Knocke, W.R., Langlais, B., Laplanche, A., Legube, B., Lykins, Jr., B., Martin, G., Martin, N., Montiel, A., Morin, M.F., Miltner, R.S., Perrine, D., Prévost, M., Reckhow, D.A., Servais, P., Singer, P.C., Sproul, O.J. and Ventresque, C. 1991b. Practical application of ozone: principles and case studies. (In) Ozone in water treatment: application and engineering. Langlais, B., Reckhow, D.A. and Brink, D.R., editors. Lewis Publishers, Chelsea, MI. pp. 133-316.

Bablon, G., Bellamy, W.D., Bourbigot, M., Daniel, F.B., Doré, M., Erb, F., Gordon, G., Langlais, B., Laplanche, A., Legube, B., Martin, G., Masschelein, W.J., Pacey, G., Reckhow, D.A. and Ventresque, C. 1991a. Fundamental aspects. (In) Ozone in water treatment: application and engineering. Langlais, B., Reckhow, D.A. and Brink, D.R., editors. Lewis Publishers, Chelsea, MI. pp. 11-132.

Bader, H. and Hoigné, J. 1981. Determination of ozone in water by the indigo method. Water Research **15**:449-456.

Bancroft, K., Chrostowski, P., Wright, R.L. and Suffet, I. 1984. Ozonation and oxidation competition values. Water Research **18**:473-478.

Bellamy, W.D., Damez, F., Langlais, B., Montiel, A., Rakness, K.L., Reckhow, D.A. and Robson, C.M. 1991. Engineering aspects. (In) Ozone in water treatment: application and engineering. Langlais, B., Reckhow, D.A., and Brink, D.R., editors. Lewis Publishers, Chelsea, MI. pp. 317-468.

Belosevic, M. 1985. Biological and immunological aspects of the host-parasite relationship in infection of mice with *Giardia muris*. PhD dissertation, Institute of Parasitology, McGill Universitiy, Montreal, Quebec. 398 pp.

Belosevic, M. and Faubert, G.M. 1983a. *Giardia muris*: correlation between oral dosage, course of infection and trophozoite distribution in mouse small intestine. Experimental Parasitology **56**:93-100.

Belosevic, M. and Faubert, G.M. 1983b. Temporal study of acquired resistance in infections of mice with *Giardia muris*. Parasitology **87**:517-524.

Belosevic, M., Faubert, G.M., Croll, N.A. and MacLean, J.D. 1982. *Giardia lamblia*: axenic growth in autoclaved and filtered Diamond's TYI-S-33 medium. Canadian Journal of Zoology **60**:1673-1675.

Belosevic, M., Faubert, G.M., MacLean, J.D., Law, C. and Croll, N.A. 1983. *Giardia lamblia* infections in Mongolian gerbils: an animal model. Journal of Infectious Diseases **147**:222-226.

Belosevic, M., Faubert, G.M., Skamene, E. and MacLean, J.D. 1984. Susceptibility and resistance of inbred mice to *Giardia muris*. Infection and Immunity **44**:282-286.

Berg, J.D. 1987. Disinfection: the physiological state of the test organism. (In) Proceedings, AWWA Seminar on Assurance of Adequate Disinfection, or C-T or Not C-T. American Water Works Association, Denver, CO. pp. 85-99.

Bifulco, J.M. and Schaefer, III, F.W. 1990. Selective concentration of *Giardia lamblia* cysts from water samples using a magnetic antibody method. (In) Proceedings of the 17th Water Quality Technology Conference. November 12-16, 1989, Philadelphia, Pennsylvania. American Water Works Association, Denver, CO. pp. 451-457.

Bingham, A.K., Jarroll, E.L., Jr. and Meyer, E.A., 1979. Induction of *Giardia* excystation and the effect of temperature on cyst viability as compared by eosin-exclusion and *in vitro* excystation. (In) Waterborne transmission of giardiasis. W. Jakubowski and J.C. Hoff, editors. United States Environmental Protection Agency, Cincinnati, OH. Report No. EPA-600/9-79-001 pp. 217-229.

Bingham, A.K. and Meyer, E.A. 1979. *Giardia* excystation can be induced *in vitro* in acidic solutions. Nature (London) **277**:301-302.

Birdsall, C.M., Jenkins, A.C. and Spadinger, E. 1952. Iodometric determination of ozone. Analytical Chemistry **22**:662-664.

Box, G.E.P., Hunter, W.G. and Hunter, J.S. 1978. Statistics for experimenters. John Wiley and Sons, New York.

Braidech, T.E. and Karlin, R.J. 1985. Causes of a waterborne giardiasis outbreak. Journal of the American Water Works Association **77**(2):48-51.

Brunet, R., Bourbigot, M. M. and Doré, M. 1984. Oxidation of organic compounds through the combination ozone-hydrogen peroxide. Ozone: Science & Engineering **6**:163-183.

Bull, R.J. and Kopfler, F.C. 1991. Health effects of disinfectants and disinfection by-products. American Water Works Association Research Foundation, Denver, CO. 192 pp.

Cerf, O. 1977. A review: tailing of survival curves of bacterial spores. Journal of Applied Bacteriology **42**:1-19.

Chang, S.D. and Singer, P.C. 1991. The impact of ozonation on particle stability and the removal of TOC and THM precursors. Journal of the American Water Works Association **83**(3):71-79.

Chelkowska, K., Grasso, D., Fábián, I. and Gordon, G. 1992. Numerical simulations of aqueous ozone decomposition. Ozone: Science & Engineering **14**:33-50.

Chick, H. 1908. An investigation of the laws of disinfection. Journal of Hygiene (Cambridge) **8**:92-158.

Cochran, W.G. 1977. Sampling techniques, 3rd edition, John Wiley & Sons New York, NY. pp. 57-59.

Coin, L., Hannoun, C. and Gomella, C. 1964. Inactivation par l'ozone du virus de la poliomyélite présent dans les eau. la Press Médicale **72**:2153-2156. (In French)

Collier, M.K. and MacDonald, P. 1983. Giardiasis - Edmonton, Alberta. Canada Diseases Weekly Report. **9-48**:189-192.

Craun, G.F. 1988. Surface water supplies and health. Journal of the American Water Works Association **80**(2):40-52.

Craun, G.F. 1990. Waterborne giardiasis. (In) Human Parasitic Diseases, Volume 3, Giardiasis. E.A. Meyer, editor. Elsevier, Amsterdam, pp. 267-293.

Craun, G.F. and McGoldrick, J.L. 1990. Workshop on methods for investigation of waterborne disease outbreaks: summary of recommendations. EPA/600/9-90/021. Health Effects Research Laboratory, United States Environmental Protection Laboratory, Cincinnati, Ohio.

Dahi, E. 1976. Physicochemical aspects of disinfection of water by means of ultrasound and ozone. Water Research **10**:677-684.

Daniel, C. 1959 Use of half-normal plots in interpreting factorial two-level experiments. Technometrics **1**(4):311-341.

Davidson, R.A. 1990. Treatment of giardiasis: the North American perspective. (In) Human Parasitic Diseases, Volume 3, Giardiasis. E.A. Meyer, editor. Elsevier, Amsterdam, pp. 325-334.

Davies, O.L., editor. 1979. The design and analysis of industrial experiments. Longmans, New York, NY.

deRegnier, D.P., Cole, L., Schupp, D.G. and Erlandsen, S.L. 1989. Viability of *Giardia* cysts suspended in lake, river, and tap water. Applied and Environmental Microbiology. **55**:1223-1229.

Doré, M., Brunet, R., Legube, B. and Croue, J.P. 1987. The role of alkalinity in the effectiveness of processes of oxidation by ozone. (In) The Role of Ozone in Water and Wastewater Treatment. Proceedings of the Second International Conference. D.W. Smith and G.R. Finch, editors. TekTran International, Kitchener, Ontario. pp. 1-14.

Duguet, J.P., Feray, C., Dumoutier, N. and Mallevialle, J. 1989. Efficacy of the combined use of ozone/uv or ozone/hydrogen peroxide for water disinfection. Proceedings of Wasser Berlin '89 held April 10-16, 1990, Berlin, Germany **5**:1-10.

Duncan, D.B. 1955. Multiple range and multiple F tests. Biometrics. **11**:1-42.

Dupont, H.L. and Sullivan, P.S. 1986. Giardiasis: the clinical spectrum, diagnosis and therapy. Pediatric Infectious Disease **5**:S130-S138.

Dykes, A.C., Juranek, D.D., Lorenz, R.A., Sinclair, S., Jakubowski, W. and Davies, R. 1980. Municipal waterborne giardiasis: an epidemiologic investigation. Annals of Internal Medicine **92**:165-170.

Engelkirk, P.G. and Pickering, L.K. 1990. Detection of *Giardia* by immunologic methods. (In) Human Parasitic Diseases, Volume 3, Giardiasis. E.A. Meyer, editor. Elsevier, Amsterdam, pp. 187-198.

Erlandsen, S. L., Sherlock, L.A., Bemrick, W.J., Ghobrial, H. and Jakubowski, W. 1990. Prevalence of *Giardia* spp. in beaver and muskrat populations in northeastern states and Minnesota: detection of intestinal trophozoites at necropsy provides greater sensitivity than detection of cysts in fecal samples. Applied and Environmental Microbiology **56**:31-36.

Erlandsen, S.L., Meyer, E.A. and Nash, T.E. 1988. Panel discussion on taxonomy of the genus *Giardia*. (In) Advances in *Giardia* Research. P.M. Wallis and B.R. Hammond, editors. The University of Calgary Press, Calgary, Alberta. pp. 287-289.

Farooq, S., Chain, E.S.K. and Engelbrecht, R.S. 1977. Basic concepts in disinfection with ozone. Journal of the Water Pollution Control Federation **49**:1818-1831.

Faubert, G.M. and Belosevic, M. 1990. Animal models for giardiasis. (In) Human Parasitic Diseases, Volume 3, Giardiasis. E.A. Meyer, editor. Elsevier, Amsterdam. pp. 77-90.

Faubert, G.M., Belosevic, M., Skamene, E. and MacLean, J.D. 1985. Giardiasis: characteristics of infection in recombinant and male and female mice. Progress in Leucocyte Biology. **3**:465-470.

Faubert, G.M., Belosevic, M., Walker, T.S., MacLean, J.D. and Meerovitch, E. 1983. Comparative studies on the pattern of infection with *Giardia* spp. in Mongolian gerbils. Journal of Parasitology. **69**:802-805.

Fawell, J.K., Fielding, M. and Ridgway, J.W. 1987. Health risks of chlorination - is there a problem? Journal of the Institution of Water & Environmental Management **1**:61-66.

Feachem, R.G., Bradley, D.J., Garelick, H., and Mara, D.D. 1983. Sanitation and disease. Health aspects of excreta and wastewater management. John Wiley & Sons, New York, NY. pp. 349-356.

Feely, D.E., Holberton, D.V., and Erlandsen, S.L. 1990. The biology of *Giardia*. (In) Human Parasitic Diseases, Volume 3, Giardiasis. E.A. Meyer, editor. Elsevier, Amsterdam, pp. 11-49.

Fenton, H.J.H. 1894. Oxidation of tartaric acid in presence of iron. Journal of the Chemical Society **65**:899-904.

Ferguson, D.W., McGuire, M.J., Koch, B., Wolfe, R.L. and Aieta, E.M. 1990. Comparing PEROXONE and ozone for the control of taste and odor compounds, disinfection by-products, and microorganisms. Journal of the American Water Works Association **82**(4):181-191.

Filice, F.P. 1952. Studies on the cytology and life history of a *Giardia* from the laboratory rat. University of California Publications in Zoology. **57**:53-146.

Finch, G.R. and Smith, D.W. 1989. Ozone dose-response of *Escherichia coli* in activated sludge effluent. Water Research **23**:1017-1025.

Finch, G.R., Smith, D.W. and Stiles, M.E. 1988. Dose-response of *Escherichia coli* in ozone demand-free phosphate buffer. Water Research **22**:1563-1570.

Finch, G.R., Yuen, W.C. and Uibel, B.J. 1992. Inactivation of *Escherichia coli* in ozone and ozone-hydrogen peroxide. Environmental Technology **13**:571-578.

Fisher, R.A., Thornton, H.G. and MacKenzie, W.A. 1922. The accuracy of the plating method of estimating the density of bacterial populations. Annals of Applied Biology **9**:325-359.

Foliguet, J.M., Hartemann, P. and Vial, J. 1987. Microbial pathogens transmitted by water. Journal of Environmental Pathology, Toxicology, and Oncology, **7(5/6)**:39-114.

Gassmann, L. and Schwartzbrod, J. 1991. Wastewater and *Giardia* cysts. Water Science and Technology **24**(2):183-186.

Gillin, F.D. 1984. The role of reducing agents and the physiology of trophozoite attachment. (In) *Giardia* and giardiasis. S.L. Erlandsen and E.A. Meyer, editors. Plenum Press, New York pp. 111-130.

Gilmour, R.A., Smith, H.V., Smith, P.G., Morris, G.P. and Girdwood, R.W.A. 1991. The occurrence and viability of *Giardia* spp. cysts in UK waters. Water Science and Technology **24**(2):179-182.

Glaze, W.H. 1986. Chemistry of ozone, by-products, and their health effects. (In) Proceedings, AWWA Seminar on Ozonation: Recent Advances and Research Needs. American Water Works Association, Denver, CO. pp. 1-15.

Glaze, W.H., Kang, J.W., and Chapin, D.H. 1987. The chemistry of water treatment processes involving ozone, hydrogen peroxide and ultraviolet radiation. Ozone: Science & Engineering **9**:335-352.

Glaze, W.H., Koga, M., Cancilla, D., Wang, K., McGuire, M.J., Liang, S., Davis, M.K., Tate, C.H., and J.J, Aieta, E.M. 1989. Evaluation of ozonation by-products from two California surface waters. Journal of the American Water Works Association **81**(8):66-73.

Glaze, W.H., Schep, R., Chauncey, W., Ruth, E.C., Zarnoch, J.J, Aieta, E.M., Tate, C.H., and McGuire, M.J. 1990. Evaluating oxidants for the removal of model taste and odor compounds. Journal of the American Water Works Association **82**(5):79-84.

Gomella, C. 1972. Ozone practices in France. Journal of the American Water Works Association **64**(1):39-45.

Gordon, G., Copper, W.J., Rice, R.G. and Pacey, G.E. 1988. Methods of measuring disinfectant residuals. Journal of the American Water Works Association **80**(9):94-108.

Gordon, G., Rakness K., Vornehm, D. and Wood, D. 1989. Limitations of the iodometric determination of ozone. Journal of the American Water Works Association **81**(6):72-76.

Grasso, D., and Weber, Jr., W.J. 1989. Mathematical interpretation of aqueous-phase ozone decomposition rates. Journal of Environmental Engineering, American Society of Civil Engineers **115**:541-559.

Gurol, M.D. and Singer, P.C. 1982. Kinetics of ozone decomposition: a dynamic approach. Environmental Science and Technology. **16**(7):377-383.

Haas, C.N. 1981. Rational approaches in the analysis of chemical disinfection kinetics. (In) Chemistry in Water Reuse, vol. 1. W.J. Cooper, editor. Ann Arbor Science, Ann Arbor, Michigan. pp. 381-399.

Haas, C.N. 1987. Inherent experimental variability. (In) Proceedings, AWWA Seminar on Assurance of Adequate Disinfection, or C-T or Not C-T. American Water Works Association, Denver, CO. pp. 67-83.

Haas, C.N. and Heller, B. 1990. Kinetics of inactivation of *Giardia lamblia* by free chlorine. Water Research **24**:233-238.

Haas, C.N. and Karra, S.B. 1984a. Kinetics of microbial inactivation by chlorine - I. Water Research **18**:1443-1449.

Haas, C.N. and Karra, S.B. 1984b. Kinetics of microbial inactivation by chlorine - II. Kinetics in the presence of chlorine demand. Water Research **18**:1451-1454.

Hale, D.C., Johnson, C.C. and Kirkham, M.D. 1985. *In vitro Giardia* cyst evaluation by fluorescent dyes. Microecology and Therapy **15**:141-148.

Hamelin, C. and Chung, Y.S. 1974. Optimal conditions for mutagenesis by ozone in *Escherichia coli* K12. Mutation Research **19**:323-329.

Harter, L., Frost, F., Grunenfelder, G., Perkins-Jones, K. and Libby, J. 1984. Giardiasis in an infant and toddler swim class. American Journal of Public Health **74**:155-156.

Healy, G.R., 1979. The presence and absence of *Giardia lamblia* in studies on parasite prevalence in the U.S.A. (In) Waterborne transmission of giardiasis. W. Jakubowski and J.C. Hoff, editors. United States Environmental Protection Agency, Cincinnati, OH. Report No. EPA-600/9-79-001 pp. 92-103.

Helsel, D.R. 1990. Less than obvious: statistical treatment of data below the detection limit. Environmental Science and Technology **24**:1766-1774.

Hibler, C.P., Hancock, C.M., Perger, L.M. Wegrzyn, J.C. and Swabby, K.D. 1987. Inactivation of *Giardia* cysts with chlorine at 0.5°C to 5.0°C. American Water Works Association Research Foundation, Denver, CO. 40 pp.

Hoff, J.C. 1978. The relationship of turbidity to disinfection of potable water. (In) Evaluation of the microbiology standards for drinking water. C.W. Hendricks, editor. Office of Drinking Water, United States Environmental Protection Agency, Washington, DC, pp. 103-117.

Hoff, J.C. 1987. Strengths and weaknesses of using C· t values to evaluate disinfection practice. (In) Proceedings, AWWA Seminar on Assurance of Adequate Disinfection, or C-T or Not C-T. American Water Works Association, Denver, CO. pp. 49-65.

Hoff, J.C. and Akin, E.W. 1986. Microbial resistance to disinfectants: mechanisms and significance. Environmental Health Perspectives **69**:7-13.

Hoff, J.C., Rice, E.W. and Schaefer, III, F.W. 1985. Comparison of animal infectivity and excystation as measures of *Giardia muris* cyst inactivation by chlorine. Applied and Environmental Microbiology **50**:1115-1117.

Hoigné, J. 1982. Mechanisms, rates and selectivities of oxidations of organic compounds initiated by ozonation of water. (In) Handbook of ozone technology and applications, volume 1. R.G. Rice and A. Netzer, editors. Ann Arbor Science Publishers, Ann Arbor, MI. pp. 341-379.

Hoigné, J. and Bader, H. 1975. Identification and kinetic properties of the oxidizing decomposition products of ozone in water and its impacts on water purification. (In) Proceedings of the Second International Symposium on Ozone Technology. May 11-14, 1975, Montreal, Canada. Ozone Press Internationale, Jamesville, NY. pp. 271-282.

Hoigné, J. and Bader, H. 1978. Ozone initiated oxidations of solutes in wastewater: a reaction kinetic approach. Progress in Water Technology **10**(5/6):657-671.

Hoigné, J. and Bader, H. 1979a. Ozonation of water: selectivity and rate of oxidation of solutes. Ozone: Science & Engineering **1**:73-85.

Hoigné, J. and Bader, H. 1979b. Ozonation of water: Oxidation-competition values of different types of waters used in Switzerland. Ozone: Science & Engineering **1**:357-372.

Hom, L.W. 1972. Kinetics of chlorine disinfection in an ecosystem. Journal of the Sanitary Engineering Division, American Society of Civil Engineers **98**(SA1):183-193.

Ilardi, I., Shiddo, S.C., Hussein, A.S., Moxamed, H.H., Bile, M.K., Sebastiani, A., Bianchini, G., and Amiconi, G. 1986. Indagine epidemiologica sulle parassitosi intestinali in una comunita' chiusa in Somalia: prevalenza di *Trichuris trichiura,* Ancylostoma e *Giardia intestinales.* Annali dell'Istituto Superiore di Sanita. **22**(1):273-276. (In Italian)

IOA. 1990. Design guidance manual for ozone systems. Dimitriou, M.A., editor. Pan American Committee, International Ozone Association, Norwalk, CT.

Ishizaki, K., Sawadaishi, K., Miura, K. and Shinriki, N. 1987. Effect of ozone on plasmid DNA of *Escherichia coli* in situ. Water Research **21**:823-827.

Iwanczuk, I. 1968. Studies on the distribution of *Lamblia muris* in the alimentary tract of white mice. Acta Parasitologica Polonica. **15**:367-373.

Jackson, P.R., Pappas, M.G. and Hansen, B.D. 1985. Fluorogenic substrate detection of viable intracellular and extracellular pathogenic protozoa. Science **227**:435-437.

Jakubowski, W. 1984. Detection of *Giardia* cysts in drinking water - state of the art. (In) *Giardia* and giardiasis. S.L. Erlandsen and E.A. Meyer, editors. Plenum Press, New York pp. 263-286.

Jakubowski, W., Sykora, J.L., Sorber, C.A., Casson, L.W. and Gavaghan, P.D. 1991. Detemining giardiasis prevalence by examination of sewage. Water Science and Technology **24**(2):173-178.

James M. Montgomery Consulting Engineers. 1985. Water treatment principles and design. Wiley-Interscience, New York.

Jarroll, E.L. 1988. Effect of disinfectants on *Giardia* cysts. CRC Critical Reviews in Environmental Controls. **18**:1-28.

Jones, K.H. and Senft, J.A. 1985. An improved method to determine cell viability by simultaneous staining with fluorescein diacetate-propidium iodide. The Journal of Histochemistry and Cytochemistry. **33**:77-79.

Joost, R.D., Jackson, L.A., and Bollyky, L.J. 1989. Optimization of ozone contactors for drinking water disinfection. (In) Proceedings of the ninth ozone world congress, volume 2. L.J. Bollyky, editor. Port City Press, Inc., New York, N.Y. pp. 551-561.

Kaplan, B. and Altmanshofer, D. 1985. *Giardia muris* adherence to intestinal epithelium - the role of specific anti-*Giardia* antibodies. Microecology and Therapy **15**:133-140.

Katzenelson, E., Kletter, B. and Shuval, H.I. 1974. Inactivation kinetics of viruses and bacteria in water by use of ozone. Journal of the American Water Works Association **66**(12):725-729.

Kirk, B.S., McNabney, R. and Wynn, C.S. 1972. Pilot plant studies of tertiary wastewater treatment with ozone. (In) Ozone in Water and Wastewater Treatment. F.L. Evans, editor. Ann Arbor Science Publishers, Ann Arbor, MI. pp. 61-82.

Kirner, J.C., Littler, J.D. and Angelo, L.A. 1978. A waterborne outbreak of giardiasis in Camas, Wash. Journal of the American Water Works Association **70**(1):35-40.

Klaassen, C.D., Amdur, M.O. and Doull, J., eds. 1986. Casarett and Doull's toxiciology: the basic science of poison, 3rd edition. Macmillan Publishing Company, New York, NY, pp. 18-22.

LeChevallier, M.W., Norton, W.D. and Lee, R.G. 1991. Occurrence of *Giardia* and *Cryptosporidium* spp. in surface water supplies. Applied and Environmental Microbiology **57**:2610-2616.

LeChevallier, M.W., Norton, W.D., and Lee, R.G. 1992. Erratum: Occurrence of *Giardia* and *Cryptosporidium* spp. in surface water supplies. Applied and Environmental Microbiology 58:780.

Lev, O. and Regli, S. 1992a. Evaluation of ozone disinfection systems: characteristic concentration C. Journal of Environmental Engineering, American Society of Civil Engineers 118(4):477-494.

Lev, O. and Regli, S. 1992b. Evaluation of ozone disinfection systems: characteristic time T. Journal of Environmental Engineering, American Society of Civil Engineers 118(2):268-285.

Levenspiel, O. 1972. Chemical reaction engineering, 2nd edition, John Wiley & Sons, New York, NY.

Levine, N.D. 1979. *Giardia lamblia*: classification, structure, identification. (In) Waterborne transmission of giardiasis. W. Jakubowski and J.C. Hoff, editors. United States Environmental Protection Agency, Cincinnati, OH. Report No. EPA-600/9-79-001 pp. 2-8.

Levine, W.C., Stephenson, W.T. and Craun, G.F. 1990. Waterborne Disease Outbreaks 1986-1988. Morbidity and Mortality Weekly Report 39(SS-1):1-13.

Lewis, P.D. 1984. Parasites of dogs and cats in Alberta. Final report to the Alberta Environmental Research Trust. 27 pp.

Lewis, P.D., Belosevic, M., Faubert, G.M., Curthroys, L. and MacLean, J.D. 1987. Cortisone induced recrudescence of *Giardia lamblia* infections in gerbils. American Journal of Tropical Medicine and Hygiene 36:33-40.

Lin, S.D. 1985. *Giardia lamblia* and water supply. Journal of the American Water Works Association 77(2):40-47.

Lippy, E.C. 1978. Tracing a giardiasis outbreak at Berlin, New Hampshire. Journal of the American Water Works Association 70(9):512-520.

Lippy, E.C. 1984. Review of treatment practices, Rossdale Water Treatment Plant, Edmonton, Alberta. Report submitted to Alberta Environment. 8 pp.

Lippy, E.C. 1986. Engineering aspects of waterborne outbreak investigation. (In) Waterborne Diseases in the United States. G.F. Craun, editor. CRC Press, Inc., Boca Raton, Florida. pp. 215-230.

Logsdon, G.S. and Hoff, J.C. 1986. Barriers to the transmission of waterborne disease. (In) Waterborne Diseases in the United States. G.F. Craun, editor. CRC Press, Boca Raton, Florida. pp. 255-274.

Madsen, T. and Nyman, M. 1907. Zur theorie der desinfektion. Zeitschrift für Hygiene und Infektionskrankheiten **57**:388-404. (In German)

Masschelein, W., Denis, M. and Ledent, R. 1977. Spectophotometric determination of residual hydrogen peroxide. Water & Sewage Works **124**(8):69-72.

Mata, L. 1986. *Cryptosporidium* and other protozoa in diarrheal disease in less developed countries. Pediatric Infectious Disease **5**:S117-S130.

McClure, S. and Mackenzie, I.B. 1988. A regulatory agency's experience with *Giardia*. (In) Advances in *Giardia* Research. P.M. Wallis and B.R. Hammond, editors. The University of Calgary Press, Calgary, Alberta. pp. 129-131.

Metropolitan Water District of Southern California and James M. Montgomery Consulting Engineers. 1991 Pilot-scale evaluation of ozone and PEROXONE. American Water Works Association Research Foundation, Denver, CO. 321 pp.

Meyer, E.A. 1990. Taxonomy and nomenclature. (In) Human Parasitic Diseases, Volume 3, Giardiasis. E.A. Meyer, editor. Elsevier, Amsterdam, pp. 51-60.

Meyer, E.A. and Jarroll, E.L. 1980. Giardiasis. Journal of Epidemiology **111**(1):1-12.

Meyer, E.A. and Radulescu, S. 1979. *Giardia* and giardiasis. (In) Advances in Parasitology, vol. 17. W.H.R. Lumsden, R. Muller and J.R. Baker, editors. Academic Press. pp. 1-47.

Meyer, E.A. and Radulescu, S. 1984. *In vitro* cultivation of *Giardia* trophozoites. (In) *Giardia* and giardiasis. S.L. Erlandsen and E.A. Meyer, editors. Plenum Press, New York pp. 99-109.

Meyer, E.A. and Schaefer, III, F.W. 1984. Models for excystation. (In) *Giardia* and giardiasis. S.L. Erlandsen and E.A. Meyer, editors. Plenum Press, New York pp. 131-144.

Meyer, E.A., 1979. The propagation of *Giardia* trophozoites *in vitro*. (In) Waterborne transmission of giardiasis. W. Jakubowski and J.C. Hoff, editors. United States Environmental Protection Agency, Cincinnati, OH. Report No. EPA-600/9-79-001 pp. 211-216.

Newton, W.L., and Jones, M.F. 1949. The effect of ozone in water on cysts of *Endamoeba histolytica*. American Journal of Tropical Medicine **29**:669-681.

Noot, D.K., Anderson, W.B., Daignault, S.A., Williams, D.T. and Huck, P.M. 1989. Evaluating treatment processes with the Ames mutagenicity assay. Journal of the American Water Works Association **81**(9):87-102.

Ohlrogge, J.B. and Kernan, T.P. 1983. Toxicity of activated oxygen: lack of dependence on membrane fatty acid composition. Biochemcal and Biophysical Research Communications **113**:301-308.

Ongerth, J.E. 1989. *Giardia* cyst concentrations in river water. Journal of the American Water Works Association **81**(9):81-90.

Ongerth, J.E., Johnson, R.L., Macdonald, S.C., Frost, F. and Stibbs, H.H. 1989. Backcountry water treatment to prevent giardiasis. American Journal of Public Health. **79**:1633-1637.

Ongerth, J.E., Riggs, J. and Crook, J. 1988. A study of water treatment practices for the removal of *Giardia lamblia* cysts. American Water Works Association Research Foundation, Denver, CO. 56 pp.

Paszko-Kolva, C., Hacker, P.A., Stewart, M.H. and Wolfe, R.L. 1992. Impact of selected operational parameters on assimilable organic carbon removal efficiencies under pilot-plant conditions. (In) Proceedings, 1992 Spring Conference, International Ozone Association, Pan American Committee, March 10-13, 1992, Pasadena, California.

Payment, P. 1989. Bacterial colonization of domestic reverse-osmosis water filtration units. Canadian Journal of Microbiology **35**:1065-1067.

Porter, J.P., Ragazzoni, H.P., Buchanon, J.D., Waskin, H.A., Juranek, D.D., and Parkin, W.E. 1988. *Giardia* transmission in a swimming pool. American Journal of Public Health **78**:659-664.

Prokop, A. and Humphrey, A.E. 1970. Kinetics of disinfection. (In) Disinfection. M.A. Bernarde, editor. Marcel Dekker, Inc., New York, NY. pp. 61-83.

Pryor, W.A., Dooley, M.M. and Church, D.F. 1983. Mechanisms for the reaction of ozone with biological molecules: the source of the toxic effects of ozone. (In) Advances in Modern Environmental Toxicology. M.G. Mustafa and M.A. Mehlman, editors. Ann Arbor Science Publishers, Ann Arbor, MI. pp. 7-19.

Rakness, K.L., Stolarik, G.F., and Christie, J.D. 1991. Operating strategy to meet SWTR disinfection regulations at the Los Angeles aqueduct filtration plant. (In) Proceedings, 1991 Pan American Committee Conference, International Ozone Association, September 16-18, 1991, Toronto, Ontario. pp. 58-77.

Regli, S. 1987. USEPA disinfection regulations. (In) Proceedings, AWWA Seminar on Assurance of Adequate Disinfection, or C-T or Not C-T. American Water Works Association, Denver, CO. pp. 1-10.

Regli, S., Rose, J.B., Haas, C.N. and Gerba, C.P. 1991. Modeling the risk from *Giardia* and viruses in drinking water. Journal of the American Water Works Association **83**(11):76-84.

Rendtorff, R.C. 1954. The experimental transmission of human intestinal protozoan parasites. II. *Giardia lamblia* cysts given in capsules. American Journal of Hygiene. **59**:209-220.

Rice, R.G. 1989. Chemistries of ozone for pool and spa water treatment - facts and fallacies. (In) Proceedings of the ninth ozone world congress, volume 2. L.J. Bollyky, editor. Port City Press, Inc., New York, N.Y. pp. 390-418.

Rice, R.G. 1991. Implementation of the U.S. safe drinking water act amendments of 1986: their impacts on the use of ozone for drinking water treatment in the United States: 1991 update. (In) Proceedings, 1991 Pan American Committee Conference, International Ozone Association, September 16-18, 1991, Toronto, Ontario. pp. 1-26.

Rice, R.G. and Gomez-Taylor, M. 1987. Oxidation byproducts from drinking water treatment. (In) Proceedings of the Second National Conference on Drinking Water. P.M. Huck and P. Toft, editors. Pergamon, Toronto. pp. 107-133.

Rice, R.G., Robson, C.M., Miller, G.W. and Hill, A.G. 1981. Uses of ozone in water treatment. Journal of the American Water Works Association **73**(1):44-57.

Richardson, K.J., Stewart, M.H. and Wolfe, R.L. 1991. Application of gene probe technology to the water industry. Journal of the American Water Works Association **83**(9):71-81.

Roberts-Thomson, I.C., Stevens, D.P., Mahmoud, A.A.F. and Warren, K.S. 1976a. Giardiasis in the mouse: an animal model. Gastroenterology **7**:57-61.

Roberts-Thomson, I.C., Stevens, D.P., Mahmoud, A.A.F. and Warren, K.S. 1976b. Acquired resistance to infection in an animal model of giardiasis. Journal of Immunology **117**:2036-2037.

Rose, J.B. and Gerba, C.P. 1991. Use of risk assessment for development of microbial standards. Water Science and Technology **24**(2):29-34.

Rose, J.B., Darbin, H. and Gerba, C.P. 1988. Correlations of the protozoa, *Cryptosporidium* and *Giardia,* with water quality variables in a watershed. Water Science and Technology **20**(11/12):271-276.

Rose, J.B., Haas, C.N. and Regli, S. 1991. Risk assessment and control of waterborne giardiasis. American Journal of Public Health **81**:709-713.

Rose, J.B., Landeen, L.K., Riley, K.R. and Gerba, C.P. 1989. Evaluation of immunofluorescence techniques for detection of *Cryptosporidium* oocysts and *Giardia* cysts from environmental samples. Applied and Environmental Microbiology **55**:3189-3196.

Ross, W.R., van Leeuwen, J. and Grabow, W.O.K. 1976. Studies on disinfection and chemical oxidation with ozone and chlorine in water reclamation. Water SA **2**:25-32.

Roth, J. A. and Sullivan, D. E. 1983. Kinetics of ozone decomposition in water. Ozone Science & Engineering **5**:37-49.

Roustan, M., Stambolieva, Z., Duguet, J.P., Wable, O. and Mallevialle, J. 1991. Research Note: Influence of hydrodynamics on *Giardia* cyst inactivation by ozone. Study by kinetics and by "CT" approach. Ozone: Science & Engineering **13**:451-462.

Roy, D. , Chian, S.K., and Engelbrecht, R.S. 1981. Kinetics of enteroviral inactivation by ozone. Journal of the Environmental Engineering Division, Proceedings of the American Society of Civil Engineers **107**(EE5):887-901.

Roy, D., Englebrecht, R.S. and Chian, E.S.K. 1982. Comparative inactivation of six enteroviruses by ozone. Journal of the American Water Works Association **74**(12):660-664.

Rubin, A.J., Evers, D.P., Eyman, C.M. and Jarroll, E.L. 1989. Inactivation of gerbil-cultured *Giardia lamblia* cysts by free chlorine. Applied and Environmental Microbiology **55**:2592-2594.

Saez, P.B. and Rittmann, R.E. 1992. Model-parameter estimation using least squares. Water Research **26**:789-796.

Sauch, J.F. 1985. Use of immunofluorescence and phase-contrast microscopy for detection and identification of *Giardia* cysts in water samples. Applied and Environmental Microbiology. **50**:1434-1438.

Sauch, J.F. 1988. A new method for excystation of *Giardia*. (In) Advances in *Giardia* Research. P.M. Wallis and B.R. Hammond, editors. The University of Calgary Press, Calgary, Alberta pp. 261-264.

Sauch, J.F. and Berman, D. 1991. Immunofluorescense and morphology of *Giardia lamblia* cysts exposed to chlorine. Applied and Environmental Microbiology **57**:1573-1575.

Sauch, J.F., Flanigan, D., Galvin, M.L., Berman, D. and Jakubowski, W. 1991. Propidium iodide as an indicator of *Giardia* cyst viability. Applied and Environmental Microbiology **57**:32643-3247.

Schaefer, III, F.W. 1990. Methods for excystation of *Giardia*. (In) Human Parasitic Diseases, Volume 3, Giardiasis. E.A. Meyer, editor. Elsevier Science Publishers, Amsterdam. pp. 111-136.

Schaefer, III, F.W., Johnson, C.H., Hsu, C.H. and Rice, E.W. 1991. Determination of *Giardia lamblia* cyst infective dose for the Mongolian gerbil (*Meriones unguiculatus*). Applied and Environmental Microbiology **57**:2408-2409.

Schaefer, III, F.W., Rice, E.W. and Hoff, J.C. 1984. Factors promoting *in vitro* excystation of *Giardia muris* cysts. Transactions of the Royal Society of Tropical Medicine and Hygiene. **78**:795-800.

Schupp, D.G. and Erlandsen, S.L. 1987a. Determination of *Giardia muris* cyst viability by differential interference contrast, phase, or brightfield microscopy. The Journal of Parasitology **73**:723-729.

Schupp, D.G. and Erlandsen, S.L. 1987b. A new method to determine *Giardia* cyst viability: correlation of fluorescein diacetate and propidium iodide staining with animal infectivity. Applied and Environmental Microbiology **53**:704-707.

Schupp, D.G., Januschka, M.M., Sherlock, L.A.F., Stibbs, H.H., Meyer, E.A., Bemrick, W.J. and Erlandsen, S.L. 1988. Production of viable *Giardia* cysts *in vitro*: determination by fluorogenic dye staining, excystation, and animal infectivity in the mouse and mongolian gerbil. Gastroenterology **95**:1-10.

Scott, D.B.M. and Lesher, E.C. 1963. Effect of ozone on survival and permeability of *Escherichia coli*. Journal of Bacteriology **85**:567-576.

Scott, K.N., Wolfe, R.L. and Stewart, M.H. 1992. Pilot-plant-scale ozone and PEROXONE disinfection of *Giardia muris* seeded into surface water supplies. Ozone: Science & Engineering **14**:71-90.

Scott, K.N., Wolfe, R.L., Stewart, M.H. and McGuire, M.J. 1989. Pilot-scale evaluation of PEROXONE and ozone for disinfection of *Giardia muris*. (In) Proceedings of the 1989 Water Quality Technology Conference held in Philadelphia, Pennsylvania. American Water Works Association, Denver, CO. pp. 709-712.

Severin, B.F. and Churn, C.C. 1987. Kinetics of microbial inactivation. (In) Proceedings, AWWA Seminar on Assurance of Adequate Disinfection, or C-T or Not C-T. American Water Works Association, Denver, CO. pp. 21-48.

Severin, B.F., Suidan, M.T. and Engelbrecht, R.S. 1984. Series-event kinetic model for chemical disinfection. Journal of Environmental Engineering, American Society of Civil Engineers **110**:430-439.

Shaw, P.K., Brodsky, R.E., Lyman, D.O., Wood B.T., Hibler, C.P., Healy, G.R., Macleod, K.I.E., Stahl, W., and Schultz, M.G. 1977. A communitywide outbreak of giardiasis with evidence of transmission by a municipal water supply. Annals of Internal Medicine **87**:426-432.

Sheffield, H.G. 1979. Ultrastructural aspects of *Giardia*. (In) Waterborne transmission of giardiasis. W. Jakubowski and J.C. Hoff, editors. United States Environmental Protection Agency, Cincinnati, OH. Report No. EPA-600/9-79-001 pp. 9-21.

Singer, P.C. 1990. Assesssing ozonation research needs in water treatment. Journal of the American Water Works Association **82**(10):78-88.

Smith, A.L. and Smith, H.V. 1989. A comparison of fluorescein diacetate and propidium iodide staining and *in vitro* excystation for determining *Giardia intestinalis* cyst viability. Parasitology. **99**:329-331.

Sobsey, M.D. 1989. Inactivation of health-related microorganisms in water by disinfection processes. Water Science and Technology, **21**(3):179-195.

Sotelo, J.L., Beltrán, F.J., Benitez, F.J. and Beltrán-Heredia, J. 1989. Henry's law constant for the ozone-water system. Water Research. **23**:1239-1246.

Sproul, O.J., Buck, C.E., Emerson, M.A., Boyce, D., Walsh, D. and Howser, D. 1979. Effect of particulates on ozone disinfection of bacteria and viruses in water. United States Environmental Protection Agency, Cincinnati, OH. Report EPA-600/2-79-089. 76 pp.

Staehelin, J. and Hoigné, J. 1985. Decomposition of ozone in water in the presence of organic solutes acting as promoters and inhibitors of radical chain reactions. Environmental Science and Technology **19**:1206-1213.

Stetzenbach, L.D., Arrowood, M.J., Marshall, M.M. and Sterling, C.R. 1988. Monoclonal antibody based immunofluorescent assay for *Giardia* and *Cryptosporidium* detection in water samples. Water Science and Technology **20**(11/12):193-198.

Sykora, J.L., Sorber, C.A., Jakubowski, W., Casson, L.W., Gavaghan, P.D., Shapiro, M.A. and Schott, M.J. 1991. Distribution of *Giardia* cysts in wastewater. Water Science and Technology **24**(2):187-192.

Sykora, J.L., States, S.J., Bancroft, W.D., Butros, S.N., Shapiro, M.A. and Conley, L.F. 1986. Monitoring of water and wastewater for *Giardia*. (In) Proceedings of the 1986 Water Quality Technology Conference held in Portland, Oregon. American Water Works Association, Denver, CO. pp. 1043-1054.

Tate, C.H. 1991. Survey of ozonation installations in North America. Journal of the American Water Works Association **83**(5):40-47.

Teefy, S.M. and Singer, P.C. 1990. Performance and analysis of tracer tests to determine compliance of a disinfection scheme with the SWTR. Journal of the American Water Works Association **82**(12):88-98.

Tombes, A.S., Landfried, S.S. and Williams, L.D.., 1979. Surface morphology of *Giardia* cysts recovered from a variety of hosts. (In) Waterborne transmission of giardiasis. W. Jakubowski and J.C. Hoff, editors. United States Environmental Protection Agency, Cincinnati, OH. Report No. EPA-600/9-79-001 pp. 22-37.

Tomiyasu, H., Fukutomi, H., and Gordon, G. 1985. Kinetics and mechanism of ozone decomposition in basic aqueous solution. Inorganic Chemistry **24**:2962-2966.

United States Department of Health and Human Services. 1986. Drinking water disinfectants. G.W. Lucier and G. Hook, editors. Environmental Health Perspectives, volume 69.

United States National Research Council. 1980. Drinking water and health, volume 2. National Academy Press, Washington, DC.

USEPA. 1989. 40 CFR parts 141 and 142 Drinking water; National primary drinking water regulations; filtration, disinfection; turbidity, *Giardia lamblia*, viruses, *Legionella*, and heterotrophic bacteria; Final rule. Federal Register **54**(124):27486-27541.

USEPA. 1991. Guidance manual for compliance with the filtration and disinfection requirements for public water systems using surface water sources. Prepared for Science and Technology Branch, Criteria and Standards Division, Office of Drinking Water, Washington, DC. American Water Works Association, Denver, CO, October 1990 draft.

Venosa, A.D. 1972. Ozone as a water and wastewater disinfectant: a literature review. (In) Ozone in Water and Wastewater Treatment. F.L. Evans, editor. Ann Arbor Science Publishers, Ann Arbor, MI. pp. 83-100.

Visvesvara, G.S., Dickerson, J.W. and Healy, G.R. 1988. Variable infectivity of human-derived *Giardia lamblia* cysts for Mongolian gerbils (*Meriones unguiculatus*). Journal of Clinical Microbiology. **26**:837-841.

Wallis, P.M. and Buchanan-Mappin, J.M. 1985. Detection of *Giardia* cysts at low concentrations in water using nuclepore membranes. Water Research **19**:331-334.

Wallis, P.M., van Roodselaar, A., Neuwirth, M., Roach, P.D., Buchanan-Mappin, J.M. and Mack, H. 1990. Inactivation of *Giardia* cysts in a pilot plant using chlorine dioxide and ozone. (In) Proceedings of the 17th Water Quality Technology Conference. November 12-16, 1989, Philadelphia, Pennsylvania. American Water Works Association, Denver, CO. pp. 695-708.

Watson, H.E. 1908. A note on the variation of the rate of disinfection with change in the concentration of the disinfectant. Journal of Hygiene (Cambridge) **8**:536-592.

Watt, R.D., Kirsch, E.J. and Grady, Jr., C.P.L. 1989. Ozone consumption kinetics in filtered activated sludge effluents: A modified approach. Water Research **23**:471-480.

Wei, J.H. and Chang, S.L. 1975. A multi-Poisson distribution model for treating disinfection data. (In) Disinfection Water and Wastewater. J.D. Johnson, editor. Ann Arbor Science, Ann Arbor, Michigan. pp.11-47.

Whiteside, C. and Hassan, H.H. 1987. Induction and inactivation of catalase and superoxide dismutase of *Escherichia coli* by ozone. Archives of Biochemistry and Biophysics **257**:464-471.

Wickramanayake, G.B. 1984. Kinetics and mechanism of ozone inactivation of protozoan cysts. PhD dissertation, Department of Civil Engineering, Ohio State University, Columbus, Ohio. 182 pp.

Wickramanayake, G.B. and Sproul, O.J. 1988. Ozone concentration and temperature effects on disinfection kinetics. Ozone: Science & Engineering **10**:123-135.

Wickramanayake, G.B., Rubin, A.J. and Sproul, O.J. 1984a. Inactivation of *Giardia lamblia* cysts with ozone. Applied and Environmental Microbiology **48**:671-672.

Wickramanayake, G.B., Rubin, A.J. and Sproul, O.J. 1984b. Inactivation of *Nægleria* and *Giardia* cysts in water by ozonation. Journal of the Water Pollution Control Federation **56**:983-988.

Wickramanayake, G.B., Rubin, A.J. and Sproul, O.J. 1985. Effects of ozone and storage temperature on *Giardia* cysts. Journal of the American Water Works Association **77**(8):74-77.

Wilkinson, L. and Leland, H. 1989. Systat: the system for statistics user's manual. Systat, Inc., Evanston, IL.

Wilson, H.S.P., Stauffer, S.J., and Walvee, T.S. 1982. Waterborne giardiasis outbreak - Alberta. Canada Diseases Weekly Report. **8-20**:97-98.

Wolfe, M.S. 1990. Clinical symptoms and diagnosis by traditional methods. (In) Human Parasitic Diseases, Volume 3, Giardiasis. E.A. Meyer, editor. Elsevier, Amsterdam, pp. 175-186.

Wolfe, R.L., Stewart, M.H., Liang, S. and McGuire, M.J. 1989a. Disinfection of model indicator organisms in a drinking water pilot plant by using PEROXONE. Applied and Environmental Microbiology **55**:2230-2241.

Wolfe, R.L., Stewart, M.H., Scott, K.N. and McGuire, M.J. 1989b. Inactivation of *Giardia muris* and indicator organisms seeded in surface water supplies by PEROXONE and ozone. Environmental Science and Technology **23**:744-745.

Yuen, W. 1990. Comparison of ozone and ozone/peroxide for disinfection of *Escherichia coli* in water. M.Sc. thesis, Department of Civil Engineering, University of Alberta, Edmonton, Alberta. 85 pp.

Yurteri, C. and Gurol, M. D. 1988. Ozone comsumption in natural waters: effects of background organic matter, pH and carbonate species. Ozone: Science & Engineering **10**:277-290.

APPENDIX A: HYDROGEN PEROXIDE DETERMINATION USING MASSCHELEIN'S METHOD

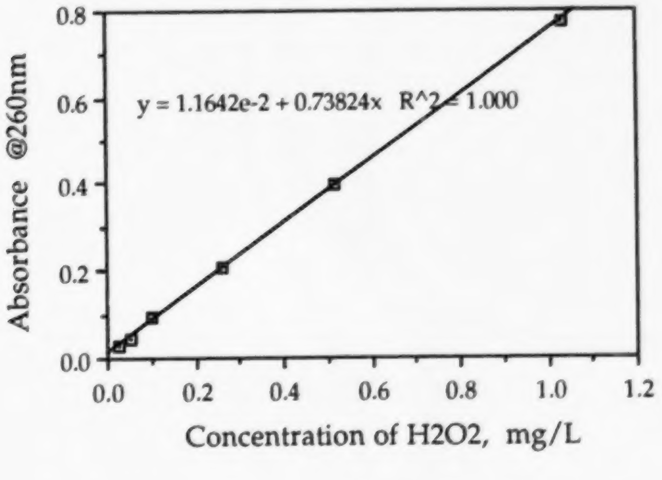


Figure A.1 Hydrogen peroxide residual calibration curve using Masschelein's method (solution 1)

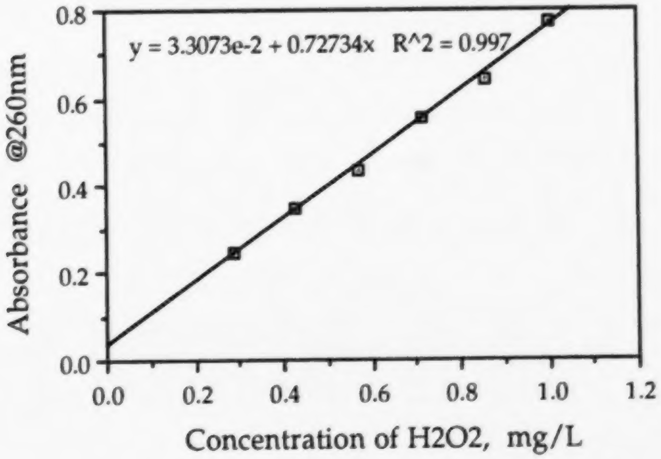


Figure A.2 Hydrogen peroxide residual calibration curve using Masschelein's method (solution 2)

The results from Masschelein's method using the two different hydrogen peroxide solutions agree within 4 percent. The overall regression equation (12 points) was $y = 0.0156+0.745x$, $R^2=0.998$.

APPENDIX B: EXAMPLE CALCULATION OF *GIARDIA MURIS* INACTIVATION USING THE MOUSE MODEL

EXAMPLE CALCULATION OF *GIARDIA MURIS* INACTIVATION USING THE MOUSE MODEL

The relation used for determining inactivation (N/N_O) from mouse model data was:

$$\frac{N}{N_o} = \frac{n \times I}{n_o \times I_o} \qquad [4.8]$$

where: n is the number of positive mice in an ozonated trial;

I is the number of infectious cysts per positive mouse after ozonation, determined using the latent period (if the inoculum had 10^5, 10^4, 10^3, 10^2, or 10 infectious cysts, mice became positive on days 3, 4, 5, 6 or 8, respectively);

n_O is the number of positive mice in the control; and

I_O is the inoculum size in the control, determined by hemocytometer count.

As an example, *G. muris* inacitvation (N/N_O) in trial A1 of experiment 33 was calculated using equation [4.8] and:

$n = 4$;

$n_O = 5$;

$I = 10$ (latent period is day 8); and

$I_O = 100{,}000$ (latent period is day 3).

thus: $$\frac{N}{N_o} = \frac{4 \times 10}{5 \times 120{,}000} = 0.000067 \text{ or } 4.2 \text{ log-units}$$

APPENDIX C: SAMPLE CALCULATION OF HOMOGENEITY CHECK USING REPLICATE HEMOCYTOMETER CYST COUNTS

Table C.1 Viability comparison experiment: Homogeneity check of replicate hemocytometer cyst count data.

	Cyst Counts Before Centrifugation (Four Replicates)				
Trial	Mean (9 squares)	S^2	D^2	Poisson† Yes / No	Concentration (cysts/mL)
A1	6.88	2.23	0.97	Yes	7.6E+03
A2	4.75	1.42	0.90	Yes	5.3E+03
B1	11.38	7.06	1.86	Yes	1.3E+04
B2	10.50	8.17	2.33	Yes	1.2E+04
C1	4.63	3.56	2.31	Yes	5.1E+03
C2	7.63	2.06	0.81	Yes	8.5E+03
C3	8.00	1.50	0.56	Yes	8.9E+03
C4	11.50	8.33	2.17	Yes	1.3E+04
D1	3.63	2.40	1.98	Yes	4.0E+03
D2	7.50	1.17	0.47	Yes	8.3E+03
D3	6.38	1.23	0.58	Yes	7.1E+03
D4	8.13	3.23	1.19	Yes	9.0E+03
AC*	7.63	1.73	0.68	Yes	8.5E+03
BC	11.13	5.40	1.46	Yes	1.2E+04
CC	7.38	2.23	0.91	Yes	8.2E+03
DC	8.88	3.23	1.09	Yes	9.9E+03

*Control - Cyst Batch A;

†χ^2 (0.05, 3) = 7.81;

S^2 is the estimate of the population variance;

D^2 is the index of dispersion determined as $[(4-1) \times S^2]$/mean.

APPENDIX D: AQUEOUS RESIDUAL OZONE DETERMINATION USING THE INDIGO TRISULPHONATE METHOD

Table D.1 Aqueous residual ozone determination using the indigo trisulphonate method.

Method	Absorbance Measured at (nm)	Absorbance (1/cm)	Concentration of Ozone (mg/L)	Percent Difference (%)
u.v.	260	1.28719	18.73	
u.v.	260	1.27228	18.51	
Indigo	600	0.18000-0.01714	19.39	+4.1
u.v.	260	1.23384	17.95	
u.v.	260	1.19855	17.43	
Indigo	600	0.18000-0.02678	18.24	+3.1
u.v.	260	1.24963	18.18	
u.v.	260	1.23305	17.94	
Indigo	600	0.17949-0.02409	18.50	+2.4
u.v.	260	1.10361	16.06	
u.v.	260	1.13188	16.47	
Indigo	600	0.17949-0.03426	17.29	+6.3
u.v.	260	1.13773	16.55	
u.v.	260	1.13087	16.45	
Indigo	600	0.17949-0.03218	17.54	+6.3
u.v.	260	1.11562	16.23	
u.v.	260	1.06317	15.47	
Indigo	600	0.17949-0.03871	16.76	+5.7

Mean percent difference (6 trials) is +4.7 %

APPENDIX E: SELECTED OZONE DECOMPOSITION RESULTS

Table E.1 - Data Set A: Half-Life Summary

50 mL Erlenmeyer Flask

0.5 mg/L O3 in O.D.F. PO4

	Applied O3, mg/L	Half-Life, s
	0.47	180
	0.52	660
Ave.	0.50	420

0.5 mg/L O3 in O.D.F. Milli-Q

	Applied O3, mg/L	Half-Life, s
	0.57	420
	0.63	600
Ave.	0.60	510

1.0 mg/L O3 in O.D.F. PO4

	Applied O3, mg/L	Half-Life, s
	1.51	340
	0.75	480
Ave.	1.13	410

1.0 mg/L O3 in O.D.F. Milli-Q

	Applied O3, mg/L	Half-Life, s
	1.01	480
	1.15	480
Ave.	1.08	480

250 mL Erlenmeyer Flask

0.5 mg/L O3 in O.D.F. PO4

	Applied O3, mg/L	Half-Life, s
	0.55	840
	0.65	780
Ave.	0.60	810

0.5 mg/L O3 in O.D.F. Milli-Q

	Applied O3, mg/L	Half-Life, s
	0.51	2640
	0.59	1620
Ave.	0.55	2130

1.0 mg/L O3 in O.D.F. PO4

	Applied O3, mg/L	Half-Life, s
	1.04	1320
	0.88	840
Ave.	0.96	1080

1.0 mg/L O3 in O.D.F. Milli-Q

	Applied O3, mg/L	Half-Life, s
	1.04	3360
	1.02	3120
Ave.	1.03	3240

2.0 L Reaction Vessel

0.5 mg/L O3 in O.D.F. PO4

	Applied O3, mg/L	Half-Life, s
	0.48	900
	0.65	1020
Ave.	0.57	960

0.5 mg/L O3 in O.D.F. Milli-Q

	Applied O3, mg/L	Half-Life, s
	0.66	5880
	0.60	6180
Ave.	0.63	6030

1.0 mg/L O3 in O.D.F. PO4

	Applied O3, mg/L	Half-Life, s
	0.99	1920
	0.92	1620
Ave.	0.96	1770

1.0 mg/L O3 in O.D.F. Milli-Q

	Applied O3, mg/L	Half-Life, s
	1.20	7500
	1.35	10490
Ave.	1.28	8995

Table E.2 ANOVA for ozone decomposition Data Set A

Source		Sum of Squares	d.f.	Mean Square	*F*-ratio
Between levels of factor	A	9,113.3	2	4,556.7	54.8*
	B	604.9	1	604.9	7.3
	C	5,878.6	1	5,878.6	70.7*
Interactions	AB	516.6	2	258.3	3.1
	AC	5,460.2	2	2,730.1	32.9*
	BC	205.0	1	205.0	2.5
Error	ABC	166.2	2	83.1	-
Total		21,944.8	11		

* $P \leq 0.05$;

A, size of ozone reactor;

B, applied ozone dose;

C, type of laboratory water.

Table E.3 Regression summary for Data Set B, assuming first-order ozone decomposition

Source	Sum of Squares	d.f.	Mean Square	*F*-ratio
Regression*	55.36	3	18.45	35.7*
Residual	6.21	12	0.52	-
Total	61.57	15		

* $P \leq 0.001$;

R^2 is 89.9 percent; R^2(adjusted) is 87.4 percent;

s is 0.7193 with 16 - 4 = 12 d.f.;

R^2, coefficient of determination;

s, standard deviation of the dependent variable about the regression line.

Table E.4 Regression coefficients for Data Set B, assuming first-order ozone decomposition

Variable	Coefficient	s.e. of coeff	t-ratio
Constant	-6.54	0.18	-36.4*
temperature x pH	0.31	0.18	1.7
pH	-1.80	0.18	-10.0*
temperature	-0.34	0.18	-1.9*

* $P \leq 0.10$;

s.e., standard error.

DATA SET B: Ozone Decomposition in 0.05M Phosphate Buffer

Trial 1 - 5°C, 0 NTU, pH 5.7, 250 mL Erlenmeyer reaction vessel

Time (s)	Absorbance (1/cm)	C (mg/L)	C/C_0 (mg/L)	log C/C_0
0	0.1221	1.78	1.000	0.000
30	0.1193	1.74	0.977	-0.010
60	0.1171	1.70	0.959	-0.018
90	0.1145	1.67	0.938	-0.028
120	0.1111	1.62	0.910	-0.041
150	0.1071	1.56	0.878	-0.057
180	0.1044	1.52	0.855	-0.068
210	0.1022	1.49	0.837	-0.077
240	0.1005	1.46	0.823	-0.085
270	0.0981	1.43	0.804	-0.095
300	0.0958	1.39	0.785	-0.105
330	0.0940	1.37	0.770	-0.113
360	0.0931	1.35	0.763	-0.118
390	0.0917	1.33	0.751	-0.124
420	0.0899	1.31	0.737	-0.133
450	0.0877	1.28	0.719	-0.143
480	0.0859	1.25	0.704	-0.153
510	0.0843	1.23	0.691	-0.161
540	0.0822	1.20	0.673	-0.172
570	0.0804	1.17	0.658	-0.182
600	0.0786	1.14	0.644	-0.191
630	0.0772	1.12	0.633	-0.199
660	0.0760	1.11	0.623	-0.206
690	0.0747	1.09	0.612	-0.213
720	0.0742	1.08	0.608	-0.216
750	0.0677	0.98	0.554	-0.256
780	0.0650	0.95	0.532	-0.274
810	0.0633	0.92	0.518	-0.285
840	0.0619	0.90	0.507	-0.295
870	0.0600	0.87	0.492	-0.308
900	0.0586	0.85	0.480	-0.318

DATA SET B: Ozone Decomposition in 0.05M Phosphate Buffer

Trial 2 - 5°C, 0 NTU, pH 5.7, 250 mL Erlenmeyer reaction vessel

Time (s)	Absorbance (1/cm)	C (mg/L)	C/C_0 (mg/L)	log C/C_0
0	0.2043	2.97	1.000	0.000
30	0.2020	2.94	0.989	-0.005
60	0.1984	2.89	0.971	-0.013
90	0.1948	2.83	0.954	-0.020
120	0.1911	2.78	0.936	-0.029
150	0.1870	2.72	0.915	-0.038
180	0.1830	2.66	0.896	-0.048
210	0.1788	2.60	0.875	-0.058
240	0.1750	2.55	0.857	-0.067
270	0.1703	2.48	0.834	-0.079
300	0.1663	2.42	0.814	-0.089
330	0.1617	2.35	0.792	-0.101
360	0.1575	2.29	0.771	-0.113
390	0.1529	2.23	0.749	-0.126
420	0.1485	2.16	0.727	-0.138
450	0.1441	2.10	0.706	-0.151
480	0.1396	2.03	0.684	-0.165
510	0.1349	1.96	0.661	-0.180
540	0.1304	1.90	0.638	-0.195
570	0.1258	1.83	0.616	-0.210
600	0.1212	1.76	0.593	-0.227
630	0.1165	1.70	0.571	-0.244
660	0.1122	1.63	0.549	-0.260
690	0.1080	1.57	0.529	-0.277
720	0.1037	1.51	0.508	-0.294
750	0.0995	1.45	0.487	-0.312

DATA SET B: Ozone Decomposition in 0.05M Phosphate Buffer

Trial 3 - 22°C, 0 NTU, pH 5.7, 250 mL Erlenmeyer reaction vessel

Time (s)	Absorbance (1/cm)	C (mg/L)	C/C_o (mg/L)	log C/C_o
0	0.1167	1.70	1.000	0.000
30	0.1148	1.67	0.984	-0.007
60	0.1120	1.63	0.960	-0.018
90	0.1091	1.59	0.935	-0.029
120	0.1061	1.54	0.909	-0.041
150	0.1033	1.50	0.885	-0.053
180	0.1001	1.46	0.858	-0.067
210	0.0970	1.41	0.832	-0.080
240	0.0938	1.37	0.804	-0.095
270	0.0906	1.32	0.777	-0.110
300	0.0876	1.27	0.751	-0.125
330	0.0844	1.23	0.724	-0.141
360	0.0814	1.18	0.698	-0.156
390	0.0784	1.14	0.672	-0.173
420	0.0752	1.09	0.644	-0.191
450	0.0720	1.05	0.617	-0.210
480	0.0688	1.00	0.590	-0.229
510	0.0655	0.95	0.561	-0.251
540	0.0625	0.91	0.535	-0.271
570	0.0589	0.86	0.505	-0.297
600	0.0556	0.81	0.476	-0.322
630	0.0524	0.76	0.449	-0.348
660	0.0493	0.72	0.423	-0.374

DATA SET B: Ozone Decomposition in 0.05M Phosphate Buffer

Trial 4 - 22°C, 0 NTU, pH 5.7, 250 mL Erlenmeyer reaction vessel

Time (s)	Absorbance (1/cm)	C (mg/L)	C/C_0 (mg/L)	$\log C/C_0$
0	0.1842	2.68	1.000	0.000
30	0.1817	2.64	0.986	-0.006
60	0.1786	2.60	0.969	-0.014
90	0.1752	2.55	0.951	-0.022
120	0.1716	2.50	0.932	-0.031
150	0.1681	2.45	0.912	-0.040
180	0.1642	2.39	0.891	-0.050
210	0.1606	2.34	0.872	-0.060
240	0.1566	2.28	0.850	-0.071
270	0.1528	2.22	0.829	-0.081
300	0.1488	2.17	0.808	-0.093
330	0.1448	2.11	0.786	-0.105
360	0.1409	2.05	0.765	-0.117
390	0.1370	1.99	0.743	-0.129
420	0.1328	1.93	0.721	-0.142
450	0.1288	1.87	0.699	-0.156
480	0.1248	1.82	0.677	-0.169
510	0.1206	1.76	0.655	-0.184
540	0.1165	1.69	0.632	-0.199
570	0.1121	1.63	0.608	-0.216
600	0.1079	1.57	0.585	-0.232
630	0.1035	1.51	0.562	-0.250
660	0.0995	1.45	0.540	-0.268
690	0.0950	1.38	0.515	-0.288
720	0.0906	1.32	0.492	-0.308
750	0.0864	1.26	0.469	-0.329
780	0.0822	1.20	0.446	-0.350
810	0.0782	1.14	0.425	-0.372
840	0.0741	1.08	0.402	-0.396
870	0.0701	1.02	0.381	-0.420
900	0.0662	0.96	0.359	-0.445
930	0.0625	0.91	0.339	-0.469
960	0.0585	0.85	0.317	-0.498

DATA SET B: Ozone Decomposition in 0.05M Phosphate Buffer

Trial 5 - 5°C, 5 NTU, pH 5.7, 250 mL Erlenmeyer reaction vessel

Time (s)	Absorbance (1/cm)	C (mg/L)	C/C_o (mg/L)	log C/C_o
0	0.2475	1.43	1.000	0.000
30	0.2463	1.41	0.987	-0.006
60	0.2457	1.40	0.981	-0.008
90	0.2437	1.37	0.960	-0.018
120	0.2427	1.36	0.951	-0.022
150	0.2411	1.33	0.934	-0.030
180	0.2401	1.32	0.924	-0.034
210	0.2379	1.29	0.902	-0.045
240	0.2363	1.26	0.886	-0.053
270	0.2346	1.24	0.868	-0.061
300	0.2327	1.21	0.849	-0.071
330	0.2310	1.19	0.831	-0.080
360	0.2286	1.15	0.807	-0.093
390	0.2263	1.12	0.783	-0.106
420	0.2240	1.08	0.760	-0.119
450	0.2214	1.05	0.734	-0.134
480	0.2185	1.00	0.703	-0.153
510	0.2159	0.97	0.677	-0.169
540	0.2175	0.99	0.694	-0.159
570	0.2100	0.88	0.617	-0.209
600	0.2077	0.85	0.594	-0.227
630	0.2048	0.80	0.564	-0.249
660	0.2027	0.77	0.542	-0.266
690	0.2106	0 89	0.623	-0.205
720	0.1964	0.68	0.478	-0.320

DATA SET B: Ozone Decomposition in 0.05M Phosphate Buffer

Trial 6 - 5°C, 5 NTU, pH 5.7, 250 mL Erlenmeyer reaction vessel

Time (s)	Absorbance (1/cm)	C (mg/L)	C/C_o (mg/L)	log C/C_o
0	0.3502	3.11	1.000	0.000
30	0.3471	3.07	0.985	-0.006
60	0.3438	3.02	0.970	-0.013
90	0.3403	2.97	0.953	-0.021
120	0.3367	2.92	0.937	-0.028
150	0.3337	2.87	0.923	-0.035
180	0.3284	2.80	0.898	-0.047
210	0.3233	2.72	0.874	-0.058
240	0.3185	2.65	0.852	-0.070
270	0.3133	2.58	0.828	-0.082
300	0.3082	2.50	0.804	-0.095
330	0.3029	2.43	0.779	-0.109
360	0.2971	2.34	0.752	-0.124
390	0.2925	2.27	0.730	-0.137
420	0.2865	2.19	0.702	-0.153
450	0.2811	2.11	0.677	-0.169
480	0.2753	2.02	0.650	-0.187
510	0.2695	1.94	0.623	-0.205
540	0.2635	1.85	0.595	-0.225
570	0.2581	1.77	0.570	-0.244
600	0.2522	1.69	0.542	-0.266
630	0.2462	1.60	0.514	-0.289

DATA SET B: Ozone Decomposition in 0.05M Phosphate Buffer

Trial 7 - 22°C, 5 NTU, pH 5.7, 250 mL Erlenmeyer reaction vessel

Time (s)	Absorbance (1/cm)	C (mg/L)	C/C_o (mg/L)	log C/C_o
0	0.2477	1.47	1.000	0.000
30	0.2433	1.41	0.957	-0.019
60	0.2399	1.36	0.923	-0.035
90	0.2355	1.30	0.880	-0.056
120	0.2329	1.26	0.854	-0.069
150	0.2292	1.20	0.817	-0.088
180	0.2262	1.16	0.787	-0.104
210	0.2232	1.12	0.758	-0.120
240	0.2196	1.07	0.723	-0.141
270	0.2168	1.02	0.695	-0.158
300	0.2139	0.98	0.666	-0.177
330	0.2113	0.94	0.641	-0.193
360	0.2077	0.89	0.605	-0.218
390	0.2055	0.86	0.583	-0.234
420	0.2026	0.82	0.555	-0.256
450	0.1996	0.77	0.525	-0.280
480	0.1967	0.73	0.497	-0.304
510	0.1944	0.70	0.474	-0.325

DATA SET B: Ozone Decomposition in 0.05M Phosphate Buffer

Trial 8 - 22°C, 5 NTU, pH 5.7, 250 mL Erlenmeyer reaction vessel

Time (s)	Absorbance (1/cm)	C (mg/L)	C/C_o (mg/L)	log C/C_o
0	0.3385	2.93	1.000	0.000
30	0.3338	2.86	0.976	-0.010
60	0.3296	2.80	0.956	-0.020
90	0.3262	2.75	0.939	-0.027
120	0.3219	2.68	0.917	-0.038
150	0.3164	2.60	0.890	-0.051
180	0.3113	2.53	0.865	-0.063
210	0.3081	2.48	0.849	-0.071
240	0.3042	2.43	0.829	-0.081
270	0.2997	2.36	0.807	-0.093
300	0.2957	2.30	0.787	-0.104
330	0.2917	2.24	0.767	-0.115
360	0.2878	2.19	0.748	-0.126
390	0.2901	2.22	0.759	-0.120
420	0.2786	2.05	0.702	-0.154
450	0.2745	1.99	0.682	-0.166
480	0.2696	1.92	0.657	-0.182
510	0.2665	1.88	0.642	-0.193
540	0.2610	1.80	0.614	-0.212
570	0.2570	1.74	0.594	-0.226
600	0.2518	1.66	0.569	-0.245
630	0.2473	1.60	0.546	-0.263
660	0.2420	1.52	0.520	-0.284
690	0.2376	1.46	0.498	-0.303
720	0.2330	1.39	0.475	-0.323
750	0.2289	1.33	0.455	-0.342
780	0.2240	1.26	0.430	-0.366
810	0.2201	1.20	0.411	-0.386
840	0.2155	1.14	0.388	-0.411
870	0.2108	1.07	0.365	-0.438
900	0.2103	1.06	0.362	-0.441
930	0.2032	0.96	0.327	-0.486
960	0.2001	0.91	0.311	-0.507

DATA SET B: Ozone Decomposition in 0.05M Phosphate Buffer

Trial 9 - 5°C, 0 NTU, pH 7.6, 250 mL Erlenmeyer reaction vessel

Time (s)	Absorbance (1/cm)	C (mg/L)	C/C_o (mg/L)	log C/C_o
0	0.1026	1.49	1.000	0.000
30	0.0948	1.38	0.924	-0.034
60	0.0889	1.29	0.866	-0.062
90	0.0833	1.21	0.812	-0.090
120	0.0784	1.14	0.765	-0.117
150	0.0737	1.07	0.718	-0.144
180	0.0695	1.01	0.678	-0.169
210	0.0647	0.94	0.631	-0.200
240	0.0614	0.89	0.599	-0.223
270	0.0580	0.84	0.565	-0.248
300	0.0548	0.80	0.534	-0.272
330	0.0518	0.75	0.505	-0.297
360	0.0491	0.71	0.479	-0.320
390	0.0464	0.67	0.452	-0.345
420	0.0437	0.64	0.426	-0.370
450	0.0414	0.60	0.404	-0.394
480	0.0394	0.57	0.384	-0.416
510	0.0370	0.54	0.361	-0.442
540	0.0353	0.51	0.344	-0.463
570	0.0335	0.49	0.326	-0.486
600	0.0318	0.46	0.310	-0.509
630	0.0303	0.44	0.295	-0.530
660	0.0288	0.42	0.280	-0.552

DATA SET B: Ozone Decomposition in 0.05M Phosphate Buffer

Trial 10 - 5°C, 0 NTU, pH 7.6, 250 mL Erlenmeyer reaction vessel

Time (s)	Absorbance (1/cm)	C (mg/L)	C/C_o (mg/L)	log C/C_o
0	0.2042	2.97	1.000	0.000
30	0.1858	2.70	0.910	-0.041
60	0.1711	2.49	0.838	-0.077
90	0.1587	2.31	0.777	-0.110
120	0.1475	2.15	0.722	-0.141
150	0.1381	2.01	0.676	-0.170
180	0.1293	1.88	0.633	-0.198
210	0.1224	1.78	0.599	-0.222
240	0.1132	1.65	0.554	-0.256
270	0.1176	1.71	0.576	-0.240
300	0.1119	1.63	0.548	-0.261
330	0.0951	1.38	0.466	-0.332
360	0.0899	1.31	0.440	-0.357
390	0.0828	1.20	0.405	-0.392
420	0.0778	1.13	0.381	-0.419
450	0.0733	1.07	0.359	-0.445
480	0.0835	1.22	0.409	-0.388
510	0.0646	0.94	0.316	-0.500
540	0.0608	0.88	0.297	-0.527

DATA SET B: Ozone Decomposition in 0.05M Phosphate Buffer

Trial 11 - 22°C, 0 NTU, pH 7.6, 250 mL Erlenmeyer reaction vessel

Time (s)	Absorbance (1/cm)	C (mg/L)	C/C_o (mg/L)	log C/C_o
0	0.1208	1.76	1.000	0.000
30	0.1114	1.62	0.923	-0.035
60	0.1037	1.51	0.858	-0.066
90	0.0966	1.41	0.800	-0.097
120	0.0905	1.32	0.749	-0.126
150	0.0852	1.24	0.706	-0.151
180	0.0804	1.17	0.666	-0.177
210	0.0762	1.11	0.631	-0.200
240	0.0723	1.05	0.599	-0.223
270	0.0688	1.00	0.569	-0.245
300	0.0650	0.95	0.538	-0.269
330	0.0613	0.89	0.508	-0.294
360	0.0582	0.85	0.482	-0.317

DATA SET B: Ozone Decomposition in 0.05M Phosphate Buffer

Trial 12 - 22°C, 0 NTU, pH 7.6, 250 mL Erlenmeyer reaction vessel

Time (s)	Absorbance (1/cm)	C (mg/L)	C/C_o (mg/L)	log C/C_o
0	0.1595	2.32	1.000	0.000
30	0.1475	2.15	0.925	-0.034
60	0.1365	1.99	0.856	-0.068
90	0.1271	1.85	0.797	-0.099
120	0.1192	1.73	0.747	-0.126
150	0.1127	1.64	0.707	-0.151
180	0.1066	1.55	0.668	-0.175
210	0.1010	1.47	0.634	-0.198
240	0.0963	1.40	0.604	-0.219
270	0.0920	1.34	0.577	-0.239
300	0.0881	1.28	0.552	-0.258
330	0.0844	1.23	0.529	-0.277
360	0.0810	1.18	0.508	-0.294
390	0.0777	1.13	0.487	-0.312
420	0.0748	1.09	0.469	-0.329
450	0.0716	1.04	0.449	-0.347
480	0.0688	1.00	0.432	-0.365
510	0.0661	0.96	0.415	-0.382

DATA SET B: Ozone Decomposition in 0.05M Phosphate Buffer

Trial 13 - 5°C, 5 NTU, pH 7.6, 250 mL Erlenmeyer reaction vessel

Time (s)	Absorbance (1/cm)	C (mg/L)	C/C_o (mg/L)	log C/C_o
0	0.2570	1.23	1.000	0.000
30	0.2509	1.14	0.927	-0.033
60	0.2457	1.07	0.866	-0.062
90	0.2413	1.00	0.814	-0.090
120	0.2378	0.95	0.772	-0.112
150	0.2339	0.89	0.726	-0.139
180	0.2300	0.84	0.680	-0.168
210	0.2270	0.79	0.644	-0.191
240	0.2242	0.75	0.612	-0.213
270	0.2219	0.72	0.585	-0.233
300	0.2197	0.69	0.559	-0.253
330	0.2171	0.65	0.528	-0.277
360	0.2152	0.62	0.505	-0.297
390	0.2130	0.59	0.479	-0.320
420	0.2110	0.56	0.455	-0.342
450	0.2092	0.53	0.434	-0.362
480	0.2078	0.51	0.417	-0.380
510	0.2062	0.49	0.399	-0.399
540	0.2044	0.46	0.377	-0.424
570	0.2034	0.45	0.365	-0.437
600	0.2018	0.43	0.346	-0.460
630	0.2003	0.40	0.329	-0.483

DATA SET B: Ozone Decomposition in 0.05M Phosphate Buffer

Trial 14 - 5°C, 5 NTU, pH 7.6, 250 mL Erlenmeyer reaction vessel

Time (s)	Absorbance (1/cm)	C (mg/L)	C/C_o (mg/L)	log C/C_o
0	0.3296	2.54	1.000	0.000
30	0.3236	2.45	0.966	-0.015
60	0.3158	2.34	0.921	-0.036
90	0.3084	2.23	0.878	-0.056
120	0.3014	2.13	0.838	-0.077
150	0.2947	2.03	0.800	-0.097
180	0.2885	1.94	0.764	-0.117
210	0.2822	1.85	0.728	-0.138
240	0.2761	1.76	0.693	-0.159
270	0.2698	1.67	0.657	-0.183
300	0.2644	1.59	0.626	-0.204
330	0.2577	1.49	0.588	-0.231
360	0.2518	1.40	0.554	-0.257
390	0.2464	1.33	0.522	-0.282
420	0.2401	1.23	0.486	-0.313
450	0.2350	1.16	0.457	-0.340
480	0.2297	1.08	0.427	-0.370
510	0.2247	1.01	0.398	-0.400
540	0.2201	0.94	0.372	-0.430
570	0.2160	0.88	0.348	-0.458

DATA SET B: Ozone Decomposition in 0.05M Phosphate Buffer

Trial 15 - 22°C, 5 NTU, pH 7.6, 250 mL Erlenmeyer reaction vessel

Time (s)	Absorbance (1/cm)	C (mg/L)	C/C_0 (mg/L)	log C/C_0
0	0.2690	1.56	1.000	0.000
30	0.2593	1.42	0.910	-0.041
60	0.2509	1.29	0.831	-0.080
90	0.2438	1.19	0.764	-0.117
120	0.2373	1.10	0.704	-0.153
150	0.2331	1.03	0.665	-0.177
180	0.2294	0.98	0.630	-0.201
210	0.2252	0.92	0.591	-0.228
240	0.2216	0.87	0.557	-0.254
270	0.2180	0.81	0.523	-0.281
300	0.2160	0.79	0.505	-0.297
330	0.2130	0.74	0.477	-0.322
360	0.2108	0.71	0.456	-0.341
390	0.2087	0.68	0.436	-0.360
420	0.2068	0.65	0.418	-0.378
450	0.2042	0.61	0.395	-0.404
480	0.2000	0.55	0.355	-0.450
510	0.1989	0.54	0.345	-0.462
540	0.1957	0.49	0.315	-0.502

DATA SET B: Ozone Decomposition in 0.05M Phosphate Buffer

Trial 16 - 22°C, 5 NTU, pH 7.6, 250 mL Erlenmeyer reaction vessel

Time (s)	Absorbance (1/cm)	C (mg/L)	C/C_0 (mg/L)	log C/C_0
0	0.3376	2.89	1.000	0.000
30	0.3145	2.55	0.884	-0.054
60	0.2970	2.30	0.796	-0.099
90	0.2839	2.11	0.730	-0.137
120	0.2737	1.96	0.678	-0.169
150	0.2651	1.83	0.635	-0.197
180	0.2585	1.74	0.601	-0.221
210	0.2522	1.65	0.570	-0.244
240	0.2473	1.57	0.545	-0.263
270	0.2416	1.49	0.516	-0.287
300	0.2370	1.43	0.494	-0.307
330	0.2324	1.36	0.470	-0.328
360	0.2286	1.30	0.451	-0.346

APPENDIX F: SUMMARY OF TREATMENT CONDITIONS IN THE EXPERIMENTAL TRIALS

Table F.1 - Treatment conditions in the viability comparison experiment

Reference		Ozone (mg/L)				Experimental Conditions					Infectivity Results										
Exp.	Trial	Applied (mg/L)	Peak Obs. (mg/L)	Residual (mg/L)	Utilized (mg/L)	Water	Temp. (°C)	Time (min)	pH	Turbidity (NTU)	No. of Mice per trial	Inoculum (cysts/mouse)	Days After Infection (No. of mice cyst pos.)							N/No (log)
													3	4	5	6	7	8	8T	
33	A1	0.62	0.47	0.3	0.32	PB	22	5	6.7	0	5	1.2E+05	0	0	2	2	2	4	4	4.2
33	A2	0.64	0.52	0.4	0.24	PB	22	5	6.7	0	5	1.2E+05	0	0	2	3	3	4	4	4.2
33	B1	0.26	0.18	0.1	0.16	PB	22	5	6.7	0	5	1.3E+05	0	0	3	5	-	-	-	3.1
33	B2	0.30	0.22	0.1	0.20	PB	22	5	6.7	0	4	1.6E+05	0	0	1	4	-	-	-	3.1
33	C1	2.55	2.14	1.3	1.25	PB	22	5	6.7	0	5	1.4E+05	0	0	0	0	1	1	1	4.4
33	C2	0.13	0.07	0.04	0.09	PB	22	0.58	6.7	0	5	1.3E+05	2	5	-	-	-	-	-	1.2
33	C3	0.27	0.17	0.2	0.07	PB	22	0.25	6.7	0	-	-	-	-	-	-	-	-	-	-
33	C4	0.13	0.07	0.1	0.03	PB	22	0.33	6.7	0	-	-	-	-	-	-	-	-	-	-
33	D1	0.28	0.15	0.2	0.08	PB	22	0.58	6.7	0	5	7.2E+04	2	2	5	-	-	-	-	2.2
33	D2	0.23	0.15	0.1	0.13	PB	22	1	6.7	0	-	-	-	-	-	-	-	-	-	-
33	D3	0.21	0.12	0.1	0.11	PB	22	2	6.7	0	-	-	-	-	-	-	-	-	-	-
33	D4	0.18	0.07	0.02	0.16	PB	22	1.53	6.7	0	-	-	-	-	-	-	-	-	-	-
33	AC	-	-	-	-	PB	22	5	6.7	0	5	1.2E+05	5	-	-	-	-	-	-	0.0
33	BC	-	-	-	-	PB	22	5	6.7	0	5	1.2E+05	5	-	-	-	-	-	-	0.0
33	CC	-	-	-	-	PB	22	5	6.7	0	5	1.6E+05	5	-	-	-	-	-	-	0.0
33	DC	-	-	-	-	PB	22	2	6.7	0	2	1.5E+05	2	-	-	-	-	-	-	0.0

Reference		Ozone (mg/L)				FDA-EB Staining Results							Excystation Results						FDA	Infect.
Exp.	Trial					Cyst Counts				Stained Cyst Total	% of Stained Cysts that are FDA-positive	N/No (log)	ECW	PET	IC	Total	% Excyst.	N/No (log)	N/No (log)	N/No (log)
		Applied (mg/L)	Peak Obs. (mg/L)	Residual (mg/L)	Utilized (mg/L)	FDA-pos.	EB-pos.	Not stained	Total											
33	A1	0.62	0.47	0.3	0.32	0	140	3	143	140	0.0	>2.1	0	0	669	669	0.0	>2.8	>2.1	4.2
33	A2	0.64	0.52	0.4	0.24	0	165	4	169	165	0.0	>2.2	0	0	546	546	0.0	>2.7	>2.2	4.2
33	B1	0.26	0.18	0.1	0.16	4	962	52	1018	966	0.41	2.3	0	1	999	1000	0.1	3.0	2.3	3.1
33	B2	0.30	0.22	0.1	0.20	1	719	26	746	720	0.14	2.7	0	0	1000	1000	0.0	>3.0	2.7	3.1
33	C1	2.55	2.14	1.3	1.25	0	566	109	675	566	0.0	>2.8	0	0	782	782	0.0	>2.9	>2.8	4.4
33	C2	0.13	0.07	0.04	0.09	332	263	90	685	595	55.8	0.2	37	252	711	1000	28.9	0.5	0.2	1.2
33	C3	0.27	0.17	0.2	0.07	533	212	108	853	745	71.5	0.1	24	171	805	1000	19.5	0.7	0.1	-
33	C4	0.13	0.07	0.1	0.03	566	195	129	890	761	74.4	0.04	168	387	445	1000	55.5	0.2	0.04	-
33	D1	0.28	0.15	0.2	0.08	109	331	13	453	440	24.8	0.5	4	11	985	1000	1.5	1.8	0.5	2.2
33	D2	0.23	0.15	0.1	0.13	122	708	17	847	830	14.7	0.7	1	7	992	1000	0.8	2.1	0.7	-
33	D3	0.21	0.12	0.1	0.11	2	689	52	743	691	0.29	2.4	1	9	990	1000	1.0	2.0	2.4	-
33	D4	0.18	0.07	0.02	0.16	197	565	15	777	762	25.9	0.5	1	3	996	1000	0.4	2.4	0.5	-
33	AC	-	-	-	-	64	18	115	197	82	78.0	0.0	877	86	37	1000	96.3	0.0	0.0	0.0
33	BC	-	-	-	-	95	30	209	334	125	76.0	0.0	881	110	9	1000	99.1	0.0	0.0	0.0
33	CC	-	-	-	-	139	32	173	344	171	81.3	0.0	882	76	42	1000	95.8	0.0	0.0	0.0
33	DC	-	-	-	-	175	45	150	370	220	79.5	0.0	831	99	70	1000	93.0	0.0	0.0	0.0

C-control, PB is 0.05 M phosphate buffer, FDA-fluorescein diacetate, EB-ethidium bromide, ECW-empty cyst walls, PET-partially excysted trophozoites, IC-intact cysts.
Peak Obs. ozone is the highest observed ozone concentration using the diode-array spectrophotometer.

Table F.2 - Treatment conditions in the screening experiment

Reference		Ozone (mg/L)				Experimental Conditions					Infectivity Results									
Exp.	Trial	Applied (mg/L)	Peak Obs. (mg/L)	Residual (mg/L)	Utilized (mg/L)	Water	Temp. (°C)	Time (min)	pH	Turbidity (NTU)	No. of Mice per trial	Inoculum Size (cysts/mouse)	Days After Infection - No. of positive mice							N/No (log)
													3	4	5	6	7	8	8T	
30	1	1.58	1.47	1.09	0.49	PB	5	2	7.6	0	5	7.4E+03	-	0	0	1	1	1	2	4.3
30	2	3.35	3.09	2.97	0.38	PB	5	2	5.7	0	5	5.0E+02	-	0	0	0	-	0	0	>4.4
30	3	1.61	1.34	1.28	0.33	PB	22	2	5.7	0	4	1.2E+04	-	0	2	4	-	-	-	2.4
30	4	2.73	2.10	1.36	1.37	PB	22	2	7.6	0	5	1.1E+04	-	0	1	1	2	2	2	3.4
30	5	1.76	1.77	1.29	0.47	PB	5	7	5.7	0	5	3.2E+04	-	0	3	4	4	5	-	3.5
30	R5	1.48	1.48	1.14	0.34	PB	5	7	5.7	0	5	8.0E+03	-	0	2	3	4	4	5	3.5
30	6	2.90	2.81	1.64	1.26	PB	5	7	7.6	0	5	2.8E+03	-	0	2	3	3	3	5	3.8
30	7	1.54	1.48	0.29	1.25	PB	22	7	7.6	0	5	3.2E+04	-	0	1	2	2	2	3	4.0
30	8	3.15	2.97	1.77	1.38	PB	22	7	5.7	0	5	6.0E+03	-	1	3	3	3	4	5	3.2
30	R8	2.88	2.94	1.95	0.93	PB	22	7	5.7	0	5	1.6E+04	-	0	2	3	3	5	5	3.1
30	9	1.60	1.38	1.21	0.39	PB	5	2	5.7	5	5	5.0E+04	-	0	2	3	4	4	5	3.7
30	10	2.88	2.60	1.95	0.93	PB	5	2	7.6	5	5	2.0E+05	-	0	1	3	3	4	4	4.4
30	R10	2.54	2.20	1.57	0.97	PB	5	2	7.6	5	5	1.2E+05	-	0	2	5	-	-	-	3.1
30	11	1.75	1.62	1.17	0.58	PB	22	2	7.6	5	5	8.4E+03	-	0	0	1	1	1	3	4.0
30	R11	1.56	1.31	0.73	0.83	PB	22	2	7.6	5	5	1.1E+05	-	0	0	0	0	0	0	>5.0
30	12	3.15	2.87	2.76	0.39	PB	22	2	5.7	5	5	4.4E+04	-	0	0	0	0	0	0	>4.3
30	13	1.56	1.09	0.55	1.01	PB	5	7	7.6	5	5	5.0E+04	-	0	1	1	-	3	5	3.8
30	14	2.71	2.47	2.22	0.49	PB	5	7	5.7	5	5	4.0E+04	-	0	0	1	1	1	3	4.1
30	15	1.46	1.43	0.83	0.63	PB	22	7	5.7	5	5	4.6E+04	-	0	3	5	-	-	-	2.5
30	16	3.27	2.61	0.94	2.33	PB	22	7	7.6	5	5	3.6E+04	-	0	0	0	-	0	0	>4.4
30	C1	-	-	-	-	PB	22	5	5.7	0	3	8.4E+02	-	2	3	-	-	-	-	0.0
30	RC1	-	-	-	-	PB	22	5	5.7	0	5	1.4E+04	-	5	-	-	-	-	-	0.0
30	C2	-	-	-	-	PB	22	5	5.7	5	5	2.0E+04	-	5	-	-	-	-	-	0.0
30	C3	-	-	-	-	PB	22	5	7.6	0	5	2.4E+04	-	5	-	-	-	-	-	0.0
30	C4	-	-	-	-	PB	22	5	7.6	5	5	2.6E+04	-	5	-	-	-	-	-	0.0
30	RC4	-	-	-	-	PB	22	5	7.6	5	5	1.1E+04	-	5	-	-	-	-	-	0.0
30	C5	-	-	-	-	PB	22	5	5.7	0	5	2.6E+04	-	5	-	-	-	-	-	0.0
30	C6	-	-	-	-	PB	22	5	5.7	5	5	3.2E+04	-	5	-	-	-	-	-	0.0
30	C7	-	-	-	-	PB	22	5	7.6	0	5	3.5E+04	-	5	-	-	-	-	-	0.0
30	C8	-	-	-	-	PB	22	5	7.6	5	5	2.4E+04	-	5	-	-	-	-	-	0.0

C-control, PB is 0.05 M phosphate buffer.

Peak Obs. ozone is the highest observed ozone concentration using the diode-array spectrophotometer.

Table F.3 - Treatment conditions in the ozone and ozone-hydrogen peroxide, specific ozone residual and G. lamblia experiments

Reference		Ozone (mg/L)				Experimental Conditions					Infectivity Results									
Exp.	Trial	Applied (mg/L)	Peak Obs. (mg/L)	Residual (mg/L)	Utilized (mg/L)	Water	Temp. (°C)	Time (min)	pH	Turbidity (NTU)	No. of Mice per trial	Inoculum Size (cysts/mouse)	Days After Infection - No. of positive mice							N/No (log)
													3	4	5	6	7	8	8T	
31	1	1.55	0.85	0.0	1.55	PBP	22	2	5.7	0	5	2.0E+04	-	0	1	2	2	2	2	2.5
31	2	1.62	0.74	0.0	1.62	PBP	22	2	5.7	0	5	5.0E+04	0	0	5	-	-	-	-	2.2
31	3	1.68	0.94	0.0	1.68	PBP	22	2	5.7	0	5	6.5E+04	1	1	5	-	-	-	-	2.2
31	4	1.58	1.44	1.4	0.18	PCB	22	2	5.7	0	5	1.1E+03	-	0	1	1	1	2	2	3.4
31	5	1.60	1.58	1.5	0.10	PCB	22	2	5.7	0	5	5.0E+04	0	1	3	4	4	4	4	3.3
31	6	1.73	1.71	1.6	0.13	PCB	22	2	5.7	0	5	4.2E+04	0	0	0	0	1	1	1	4.9
31	C1	-	-	-	-	PBP	22	2	5.7	0	5	1.4E+04	-	5	-	-	-	-	-	0.0
31	C2	-	-	-	-	PBP	22	2	5.7	0	5	1.5E+05	5	-	-	-	-	-	-	0.0
31	C3	-	-	-	-	PCB	22	2	5.7	0	5	1.1E+04	-	5	-	-	-	-	-	0.0
31	C4	-	-	-	-	PCB	22	2	5.7	0	4	1.6E+05	4	-	-	-	-	-	-	0.0

Reference		Ozone (mg/L)				Experimental Conditions					Infectivity Results									
Exp.	Trial	Applied (mg/L)	Peak Obs. (mg/L)	Residual (mg/L)	Utilized (mg/L)	Water	Temp. (°C)	Time (min)	pH	Turbidity (NTU)	No. of Mice per trial	Inoculum Size (cysts/mouse)	Days After Infection - No. of positive mice							N/No (log)
													3	4	5	6	7	8	8T	
36	1	0.59	0.46	0.35	0.24	PB	5	5	5.7	0	4	6.6E+04	0	2	4	-	-	-	-	2.1
36	2	0.59	0.51	0.35	0.24	PB	22	5	5.7	0	5	9.4E+03	0	0	0	0	0	0	0	>5.1
36	3	0.73	0.57	0.40	0.33	PB	5	5	7.6	0	4	1.0E+05	0	0	0	1	2	2	4	4.5
36	4	0.82	0.70	0.36	0.46	PB	22	5	7.6	0	5	6.4E+04	0	0	0	0	0	0	0	>5.1
36	5	0.66	0.54	0.37	0.29	PB	14	5	6.7	0	5	1.1E+05	0	0	4	4	5	5	-	3.6
36	6	0.71	0.57	0.37	0.34	PB	15	5	6.7	0	5	1.4E+05	0	0	1	4	5	5	-	3.6
36	C	-	-	-	-	PB	22	5	7.6	0	5	1.3E+05	5	-	-	-	-	-	-	0.0
41	1	0.42	0.40	0.39	0.03	PB	5	1	5.7	0	5	1.9E+04	0	1	4	5	-	-	-	3.1
41	2	0.43	0.40	0.40	0.03	PB	5	1	7.6	0	5	8.6E+04	0	0	2	5	-	-	-	3.1
41	3	0.47	0.39	0.38	0.09	PB	5	2	7.6	0	5	8.4E+04	0	0	1	1	3	3	3	3.8
41	4	0.48	0.42	0.41	0.07	PB	14	2	6.7	0	5	1.0E+05	0	2	4	5	-	-	-	3.1
41	5	0.77	0.77	0.42	0.35	PB	14	7	6.7	0	5	1.0E+05	0	0	1	2	2	2	2	3.5
41	6	0.53	0.48	0.41	0.12	PB	22	2	5.7	0	5	9.2E+04	0	0	2	4	4	4	4	3.2
41	C	-	-	-	-	PB	22	2	6.7	0	4	1.3E+05	4	-	-	-	-	-	-	0.0

Reference		Ozone (mg/L)				Experimental Conditions					Infectivity Results							
Exp.	Trial	Applied (mg/L)	Peak Obs. (mg/L)	Residual (mg/L)	Utilized (mg/L)	Water	Temp. (°C)	Time (min)	pH	Turbidity (NTU)	Gerbils per trial	Inoculum Size (cysts/gerbil)	Days After Infection - No. of positive gerbils					
													10	11	12	13	14	14 trophozoites
43	1	1.44	1.38	0.84	0.60	PB	22	5	6.8	0	5	7.8E+04	0	0	0	0	0	0
43	2	0.95	0.78	0.53	0.42	PB	22	5	6.8	0	5	1.7E+04	0	0	0	0	0	0
43	3	0.54	0.34	0.20	0.34	PB	22	2	6.8	0	4	2.0E+04	1	1	1	1	1	1
43	C	-	-	-	-	PB	22	5	6.8	0	5	6.2E+04	1	5	-	-	-	-

C-control, PBP is 0.05 M phosphate buffer with 10:1 peroxide to ozone ratio, PCB is 0.05 M phosphate-0.01 M carbonate buffer, PB is 0.05 M phosphate buffer.
Peak Obs. ozone is the highest observed ozone concentration using the diode-array spectrophotometer.

Table F.4 - Treatment conditions in the natural waters experiment

Reference			Ozone (mg/L)			Experimental Conditions						Infectivity Results									
Exp.	Trial	Date	Applied (mg/L)	Residua (mg/L)	Utilized (mg/L)	Scale	Water	Temp. (°C)	Time (min)	pH	Turbidity (NTU)	No. of Mice per trial	Inoculum Size (cysts/mouse)	Days After Infection - No. of positive mice							N/No (log)
														3	4	5	6	7	8	8T	
39A	1	14-01-91	0.96	0.25	0.71	1	NSR	5	2	8.3	1.5	5	5.4E+04	0	0	1	1	1	1	1	2.7
39A	2	14-01-91	2.58	0.70	1.88	1	NSR	22	2	8.3	1.5	4	2.4E+05	0	0	0	1	1	1	1	3.7
39A	3	14-01-91	2.70	0.83	1.87	1	NSR	5	5	8.3	1.5	5	7.2E+04	0	0	0	0	0	0	0	>5.0
39A	4	14-01-91	3.03	0.24	2.79	1	NSR	22	5	8.3	1.5	5	2.2E+05	0	4	5	-	-	-	-	2
39A	C	14-01-91	-	-	-	1	NSR	22	5	8.3	1.5	5	9.6E+04	5	-	-	-	-	-	-	0.0
39B	1	13-02-91	0.81	0.16	0.65	1	NSR	1	2	8.1	2.6	5	1.6E+05	0	0	1	4	5	-	-	3.7
39B	2	13-02-91	2.30	0.67	1.63	1	NSR	1	5	8.1	2.6	5	1.3E+05	0	0	0	1	1	2	2	4.6
39B	3	13-02-91	1.71	0.30	1.41	1	NSR	1	5	8.1	2.6	5	9.8E+03	0	0	0	0	0	0	0	>5.2
39B	4	13-02-91	1.67	0.69	0.98	1	NSR	1	2	8.1	2.6	5	3.3E+05	0	2	3	5	-	-	-	3.2
39B	C	13-02-91	-	-	-	1	NSR	1	2	8.1	2.6	4	1.7E+05	4	-	-	-	-	-	-	0.0
39C	1	22-02-91	2.10	0.65	1.45	1	GVRD	5	5	6.3	3.8	4	5.7E+04	0	0	1	3	3	3	4	4.1
39C	2	22-02-91	1.09	0.15	0.94	1	GVRD	5	2	6.3	3.8	5	1.5E+05	0	2	5	-	-	-	-	2.1
39C	3	22-02-91	2.62	0.35	2.27	1	GVRD	22	5	6.3	3.8	5	2.5E+05	0	0	0	0	0	0	0	>5.1
39C	4	22-02-91	2.37	0.83	1.54	1	GVRD	22	2	6.3	3.8	5	6.6E+04	0	0	4	5	-	-	-	3.1
39C	C	22-02-91	-	-	-	1	GVRD	22	2	6.3	3.8	4	1.2E+05	4	-	-	-	-	-	-	0.0
39D	1	13-03-91	1.28	0.37	0.91	1	GVRD	5	4	6.3	3.9	5	2.8E+04	0	0	0	1	1	1	1	3.1
39D	2	13-03-91	1.66	0.76	0.90	1	GVRD	5	4	6.3	3.9	5	5.4E+04	0	0	0	2	2	2	2	3.1
39D	3	13-03-91	2.05	0.77	1.28	1	GVRD	5	10	6.3	3.9	5	1.0E+04	0	0	0	4	4	5	-	3.0
39D	4	13-03-91	1.71	0.34	1.37	1	GVRD	5	10	6.3	3.9	4	3.6E+04	0	0	0	1	1	1	1	3.3
39D	C	13-03-91	-	-	-	1	GVRD	5	4	6.3	3.9	5	1.2E+04	2	5	-	-	-	-	-	0.0
42	A	14-11-90	1.68	0.70	0.98	2	NSR	22	6.3	8.2	6.0	4	1.0E+04	-	0	0	0	1	1	1	3.1
42	B	14-11-90	0.89	0.38	0.51	2	NSR	22	3.2	8.2	6.0	4	1.0E+04	-	0	1	1	1	1	2	1.6
42	C	14-11-90	-	-	-	2	NSR	22	-	8.2	6.0	3	1.0E+04	-	3	-	-	-	-	-	0.0
40	1	31-01-91	1.47	0.21	1.26	2	NSR	22	4	8.3	3.0	5	1.0E+05	0	0	1	1	1	1	1	2.7
40	2	31-01-91	2.44	0.35	2.09	2	NSR	22	6	8.3	3.0	4	1.0E+05	0	0	0	1	1	1	1	3.6
40	3	31-01-91	3.67	0.80	2.87	2	NSR	22	10	8.3	3.0	4	1.0E+05	0	0	0	0	0	0	0	>5.0
40	4	31-01-91	4.95	1.23	3.72	2	NSR	22	13.5	8.3	3.0	5	1.0E+05	0	0	0	0	0	0	0	>5.0
40	C	31-01-91	-	-	-	2	NSR	22	-	8.3	3.0	4	1.0E+05	4	4	-	-	-	-	-	0.0

C-control, NSR-North Saskatchewan River, GVRD-Greater Vancouver Regional District; Scale-1, bench-scale, 2, pilot-scale.

Table F.5 - Treatment conditions for heterotrophic plate count (HPC) bacteria at bench- and pilot-scale

Exp.&Trial	Water&Date	Scale	HPCkill	G.muriskill	App.O3	DOC	O3:DOC	pH	Turb	Temp	Conduct.	Cont.Time	Res.O3
42B	NSR141190	2		1.6	0.89	2.46	0.36	8.2	6.0	22	325	3.2	0.38
42A		2		3.1	1.68	2.46	0.68	8.2	6.0	22	325	6.3	0.7
39A1	NSR140191	1		2.7	0.96	0.99	0.97	8.3	1.5	5	310	2.0	0.25
39A2		1		3.7	2.58	0.99	2.61	8.3	1.5	22	310	2.0	0.70
39A3		1		>5	2.70	0.99	2.73	8.3	1.5	5	310	5.0	0.83
39A4		1		2.0	3.03	0.99	3.06	8.3	1.5	22	310	5.0	0.24
40-C	NSR310191	2			0.00	1.62	0.00	8.3	3.0	22	280	0.3	0.00
40-1		2		2.7	1.47	1.62	0.91	8.3	3.0	22	280	4.0	0.21
40-2		2		3.6	2.44	1.62	1.51	8.3	3.0	22	280	6.0	0.35
40-3		2		>5	3.67	1.62	2.27	8.3	3.0	22	280	10.0	0.80
40-4		2		>5	4.95	1.62	3.06	8.3	3.0	22	280	13.5	1.23
44-C	NSR130291	2	0.00		0.00	1.61	0.00	8.1	2.6	11	265	0.0	0.00
44-P3		2	0.00		0.14	1.61	0.09	8.1	2.6	11	265	1.8	0.00
44-P5		2	0.45		0.28	1.61	0.17	8.1	2.6	11	265	3.6	0.02
44-P6		2	1.08		0.34	1.61	0.21	8.1	2.6	11	265	4.4	0.05
44-P7		2	1.21		0.45	1.61	0.28	8.1	2.6	11	265	5.7	0.09
39B1		1		3.7	0.81	1.61	0.50	8.1	2.6	1	300	2.0	0.16
39B2		1		4.6	2.30	1.61	1.43	8.1	2.6	1	300	5.0	0.67
39B3		1		>5.2	1.71	1.61	1.06	8.1	2.6	1	300	5.0	0.30
39B4		1		3.2	1.67	1.61	1.04	8.1	2.6	1	300	2.0	0.69
45-1	NSR270291	1	1.84		0.22	1.20	0.18	8.0	13.0	22	298	0.3	0.00
45-2		1	2.23		0.22	1.20	0.18	8.0	1.5	22	298	0.3	0.01
45-3		1	1.93		0.21	1.20	0.18	7.5	6.7	22	298	0.3	0.00
45-4		1	1.76		0.22	1.20	0.18	7.0	14.0	22	298	0.3	0.00
45-5		1	2.20		0.22	1.20	0.18	7.5	6.8	22	298	0.3	0.00
45-6		1	2.17		0.22	1.20	0.18	7.0	1.3	22	298	0.3	0.00
45-7		1	1.23		0.21	1.20	0.18	8.0	1.3	22	298	0.5	0.00
45-8		1	1.56		0.22	1.20	0.18	8.1	7.3	22	298	0.5	0.00
45-9		1	1.42		0.22	1.20	0.18	8.1	7.2	22	298	0.5	0.00
45-10		1	1.87		0.21	1.20	0.18	8.0	1.3	22	298	0.5	0.00
45-11		1	1.23		0.22	1.20	0.18	8.0	20.0	22	298	0.5	0.00

Table F.5 - Treatment conditions for heterotrophic plate count (HPC) bacteria at bench- and pilot-scale, continued

Exp.&Trial	Water&Date	Scale	HPCkill	G.muriskill	App.O3	DOC	O3:DOC	pH	Turb	Temp	Conduct.	Cont.Time	Res.O3
45-12	NSR270291	1	1.74		0.21	1.20	0.18	8.0	19.0	22	298	0.5	0.00
45-13		1	1.85		0.22	1.20	0.19	8.0	1.5	22	298	0.5	0.00
45-14		1	0.17		0.04	1.20	0.04	8.0	1.5	22	298	0.5	0.00
45-15		1	2.64		0.23	1.20	0.19	7.0	1.5	22	298	0.5	0.00
45-16		1	0.12		0.04	1.20	0.04	7.0	1.5	22	298	0.5	0.00
45-17		1	0.37		0.14	1.20	0.11	7.5	1.5	22	298	0.5	0.00
45-18		1	0.33		0.13	1.20	0.11	7.5	1.5	22	298	0.5	0.00
45-20		1	1.06		0.05	1.20	0.04	8.0	14.0	22	298	0.5	0.00
45-21		1	1.23		0.23	1.20	0.19	8.0	2.5	22	298	0.5	0.00
45-23		1	0.30		0.13	1.20	0.11	8.0	6.4	22	298	0.5	0.00
45-24		1	0.32		0.13	1.20	0.11	8.0	6.4	22	298	0.5	0.00
45-25		1	1.25		0.22	1.20	0.18	8.0	14.0	22	298	0.5	0.00
46-7	NSR220491	1	2.09		0.84	2.96	0.28	8.0	9.3	22	315	0.5	0.00
46-8		1	2.82		0.86	2.22	0.39	8.0	7.1	22	315	0.5	0.11
46-10		1	2.30		0.86	2.22	0.39	8.0	6.9	22	315	0.5	0.08
46-13		1	3.06		1.42	2.10	0.68	8.1	14.0	22	315	0.5	0.21
46-14		1	3.17		1.42	2.88	0.49	8.1	20.0	22	315	0.5	0.08
46-15		1	3.65		1.47	1.34	1.10	7.9	7.8	4	315	0.5	0.86
46-16		1	3.48		1.45	2.10	0.69	8.0	14.0	22	315	0.5	0.25
46-18		1	3.21		1.55	1.34	1.16	7.9	8.5	22	315	0.5	0.58
39C1	GVRD220291	1		4.1	2.10	9.15	0.23	6.3	3.8	5	10	5.0	0.65
39C2		1		2.1	1.09	9.15	0.12	6.3	3.8	5	10	2.0	0.15
39C3		1		>5.1	2.62	9.15	0.29	6.3	3.8	22	10	5.0	0.35
39C4		1		3.1	2.37	9.15	0.26	6.3	3.8	22	10	2.0	0.83
39D1	GVRD130391	1		3.1	1.28	9.15	0.14	6.3	3.9	5	10	4.0	0.37
39D2		1		3.1	1.66	9.15	0.18	6.3	3.9	5	10	4.0	0.76
39D3		1		3.0	2.05	9.15	0.22	6.3	3.9	5	10	10.0	0.77
39D4		1		3.3	1.71	9.15	0.19	6.3	3.9	5	10	10.0	0.34

C-control, NSR-North Saskatchewan River, GVRD-Greater Vancouver Regional District; Scale-1, bench-scale, 2, pilot-scale.

APPENDIX G: HEMOCYTOMETER COUNT DATA AND ANIMAL RESPONSES IN THE VIABILITY COMPARISON EXPERIMENT

Table G.1 Viability comparison experiment: Ozone inactivation of *G. muris* as estimated by fluorescein diacetate-ethidium bromide staining (pH 6.7; 22°C).

Trial	Cyst Counts				Stained Cyst Total	Stained Cysts that are FDA-positive(%)	Inactivation (log N/N_O)†
	FDA-positive	EB-positive	Un-stained	Total			
A1	0	140	3	143	140	0.0	>2.1
A2	0	165	4	169	165	0.0	>2.2
B1	4	962	52	1018	966	0.41	2.3
B2	1	719	26	746	720	0.14	2.7
C1	0	566	109	675	566	0.0	>2.8
C2	332	263	90	685	595	55.8	0.2
C3	533	212	108	853	745	71.5	0.006
C4	566	195	129	890	761	74.4	0.004
D1	109	331	13	453	440	24.8	0.5
D2	122	708	17	847	830	14.7	0.7
D3	2	689	52	743	691	0.29	2.4
D4	197	565	15	777	762	25.9	0.5
AC*	64	18	115	197	82	78.0	-
BC	95	30	209	334	125	76.0	-
CC	139	32	173	344	171	81.3	-
DC	175	45	150	370	220	79.5	-

† >, detection limit;

* control, cyst batch A;

FDA, fluorescein diacetate; EB, ethidium bromide.

Table G.2 Viability comparison experiment: Ozone inactivation of *G. muris* as estimated by *in vitro* excystation (pH 6.7; 22°C).

	Cyst Counts				% excyst-	Inactivation
Trial	ECW	PET	IC	Total	ation	(log N/N_O)[†]
A1	0	0	669	669	0.0	>2.8
A2	0	0	546	546	0.0	>2.7
B1	0	1	999	1000	0.1	3.0
B2	0	0	1000	1000	0.0	>3.0
C1	0	0	782	782	0.0	>2.9
C2	37	252	711	1000	28.9	0.5
C3	24	171	805	1000	19.5	0.7
C4	168	387	445	1000	55.5	0.2
D1	4	11	985	1000	1.5	1.8
D2	1	7	992	1000	0.8	2.1
D3	1	9	990	1000	1.0	2.0
D4	1	3	996	1000	0.4	2.4
AC[*]	877	86	37	1000	96.3	-
BC	881	110	9	1000	99.1	-
CC	882	76	42	1000	95.8	-
DC	831	99	70	1000	93.0	-

[†] >, detection limit;

[*] control, cyst batch A;

ECW, empty cyst walls; PET, partially excysted trophozoites; IC, intact cysts.

Table G.3 Viability comparison experiment: Number of C3H/HeN mice infected with *G. muris* as a function of days after infection and inoculum size

Trial	No. of mice in trial	Inoculum (cysts per mouse)	Days After Infection† (No. of mice positive for cysts)						Inactivation ($\log N/N_0$)
			3	4	5	6	7	8	
A1	5	1.2E+05	0	0	2	2	2	4	4.2
A2	5	1.2E+05	0	0	2	3	3	4	4.2
B1	5	1.3E+05	0	0	3	5	-	-	3.1
B2	4	1.6E+05	0	0	1	4	-	-	3.1
C1	5	1.4E+05	0	0	0	0	1	1	4.4
C2	5	1.3E+05	2	5	-	-	-	-	1.2
D1	5	7.2E+04	2	2	5	-	-	-	2.2
AC*	5	1.2E+05	5	-	-	-	-	-	-
BC	5	1.2E+05	5	-	-	-	-	-	-
CC	5	1.6E+05	5	-	-	-	-	-	-
DC	2	1.5E+05	2	-	-	-	-	-	-

† Data collected until day 8 or when all mice in trial became positive. No trophozoites were detected on day 8 in mice that were negative for cysts.

* control, cyst batch A.

APPENDIX H: SUMMARY OF ANIMAL RESPONSES IN THE EXPERIMENTAL TRIALS

Table H.1 Ozone and ozone hydrogen-peroxide experiment: Number of C3H/HeN mice infected with *G. muris* as a function of days after infection and inoculum size

Trial	Residual Ozone at 2 min (mg/L)	Buffer	Inoculum (cysts per mouse)	Days After Infection (No. of mice positive for cysts)							Inactivation (log N/N_0)
				3	4	5	6	7	8	8T#	
1	0.0	PBP	2.0E+04	-	0	1	2	2	2	2	2.5
2	0.0	PBP	5.0E+04	0	0	5	-	-	-	-	2.2
3	0.0	PBP	6.5E+04	1	1	5	-	-	-	-	2.2
4	1.4	PCB	1.1E+03	-	0	1	1	1	2	2	3.4
5	1.5	PCB	5.0E+04	0	1	3	4	4	4	4	3.3
6	1.6	PCB	4.2E+04	0	0	0	0	1	1	1	4.9
C1*	-	PBP	1.4E+04	-	5	-	-	-	-	-	0.0
C2	-	PBP	1.5E+05	5	-	-	-	-	-	-	0.0
C3	-	PCB	1.1E+04	-	5	-	-	-	-	-	0.0
C4	-	PCB	1.6E+05	4†	-	-	-	-	-	-	0.0

*C1, control 1;

#8T, number of mice positive for cysts or trophozoites on day 8 out of cohort of 5;

†4, cohort was 4 mice in this trial;

PBP, 0.05 M phosphate buffer with 10 to 1 peroxide to ozone ratio;

PCB, 0.05 M phosphate-0.01 M carbonate buffer.

Table H.2 Screening experiment: Number of C3H/HeN mice infected with *G. muris* as a function of days after infection and inoculum size

Trial	Inoculum (cysts per mouse)	No. of mice per trial	Days After Infection (Number of mice positive for cysts)						Inactivation (log N/N_0)†
			4	5	6	7	8	8T#	
1	7.4E+03	5	0	0	1	1	1	2	4.3
2	5.0E+02	5	0	0	0	-	0	0	>4.4
3	1.2E+04	4	0	2	4	-	-	-	2.4
4	1.1E+04	5	0	1	1	2	2	2	3.4
5	3.2E+04	5	0	3	4	4	5	-	3.5
R5	8.0E+03	5	0	2	3	4	4	5	3.5
6	2.8E+03	5	0	2	3	3	3	5	3.8
7	3.2E+04	5	0	1	2	2	2	3	4.0
8	6.0E+03	5	1	3	3	3	4	5	3.2
R8	1.6E+04	5	0	2	3	3	5	5	3.1
9	5.0E+04	5	0	2	3	4	4	5	3.7
10	2.0E+05	5	0	1	3	3	4	4	4.4
R10	1.2E+05	5	0	3	5	-	-	-	3.1
11	8.4E+03	5	0	0	1	1	1	3	4.0
R11	1.1E+05	5	0	0	0	0	0	0	>5.0
12	4.4E+04	5	0	0	0	0	0	0	>4.3
13	5.0E+04	5	0	1	1	-	3	5	3.8
14	4.0E+04	5	0	0	1	1	1	3	4.1
15	4.6E+04	5	0	3	5	-	-	-	2.5
16	3.6E+04	5	0	0	0	-	0	0	>4.4
C1	8.4E+02	3	2	3	-	-	-	-	0.0
RC1*	1.4E+04	5	5	-	-	-	-	-	0.0
C2	2.0E+04	5	5	-	-	-	-	-	0.0
C3	2.4E+04	5	5	-	-	-	-	-	0.0
C4	2.6E+04	5	5	-	-	-	-	-	0.0
RC4	1.1E+04	5	5	-	-	-	-	-	0.0
C5	2.6E+04	5	5	-	-	-	-	-	0.0
C6	3.2E+04	5	5	-	-	-	-	-	0.0
C7	3.5E+04	5	5	-	-	-	-	-	0.0
C8	2.4E+04	5	5	-	-	-	-	-	0.0

† >, detection limit;

*RC1, repeat of control 1;

#8T, number of mice positive for cysts or trophozoites on day 8.

Table H.3 Specific ozone residual experiment: Number of C3H/HeN mice infected with *G. muris* as a function of days after infection and inoculum size

Trial	Inoculum (cysts per mouse)	No. of mice per trial	Days After Infection (Number of mice positive for cysts)							Inactivation (log N/N_0)[†]
			3	4	5	6	7	8	8T[#]	
1	6.6E+04	4	0	2	4	-	-	-	-	2.1
2	9.4E+03	5	0	0	0	0	0	0	0	>5.1
3	1.0E+05	4	0	0	0	1	2	2	4	4.5
4	6.4E+04	5	0	0	0	0	0	0	0	>5.1
5	1.1E+05	5	0	0	4	4	5	5	-	3.6
R5	1.4E+05	5	0	0	1	4	5	5	-	3.6
C1[*]	1.3E+05	5	5	-	-	-	-	-	-	0.0
6	1.2E+05	5	0	0	2	2	2	4	4	4.2
R6	1.2E+05	5	0	0	2	3	3	4	4	4.2
C2	1.2E+05	5	5	-	-	-	-	-	-	0.0
7	3.2E+04	5	-	0	1	2	2	2	3	4.0
C3	2.4E+04	5	-	5	-	-	-	-	-	0.0
8	1.0E+05	5	0	0	1	2	2	2	2	3.5
9	8.4E+04	5	0	0	1	1	3	3	3	3.8
10	1.0E+05	5	0	2	4	5	-	-	-	3.1
11	9.2E+04	5	0	0	2	4	4	4	4	3.2
12	1.9E+04	5	0	1	4	5	-	-	-	3.1
13	8.6E+04	5	0	0	2	5	-	-	-	3.1
C4	1.3E+05	4	4	-	-	-	-	-	-	0.0
14	7.2E+04	5	2	2	5	-	-	-	-	2.2
C5	1.5E+05	2	2	-	-	-	-	-	-	0.0

[†] >, detection limit;

[*] C1, control 1;

[#] 8T, number of mice positive for cysts or trophozoites on day 8.

Table H.4 Natural waters experiment: Number of C3H/HeN mice infected with *G. muris* as a function of days after infection and inoculum size

Trial	Water	Inoculum (cysts per mouse)	No. of mice per trial	Days After Infection (No. of mice positive for cysts)							Inactivation (log N/N_0)†
				3	4	5	6	7	8	8T#	
1	NSR	5.4E+04	5	0	0	1	1	1	1	1	2.7
2	NSR	2.4E+05	4	0	0	0	1	1	1	1	3.7
3	NSR	7.2E+04	5	0	0	0	0	0	0	0	>5.0
4	NSR	2.2E+05	5	0	4	5	-	-	-	-	2
39AC*	NSR	9.6E+04	5	5	-	-	-	-	-	-	0.0
5	GVRD	5.7E+04	4	0	0	1	3	3	3	4	4.1
6	GVRD	1.5E+05	5	0	2	5	-	-	-	-	2.1
7	GVRD	2.5E+05	5	0	0	0	0	0	0	0	>5.1
8	GVRD	6.6E+04	5	0	0	4	5	-	-	-	3.1
39CC	GVRD	1.2E+05	4	4	-	-	-	-	-	-	0.0
9	GVRD	2.8E+04	5	0	0	0	1	1	1	1	3.1
10	GVRD	5.4E+04	5	0	0	0	2	2	2	2	3.1
11	GVRD	1.0E+04	5	0	0	0	4	4	5	-	3.0
12	GVRD	3.6E+04	4	0	0	0	1	1	1	1	3.3
39DC	GVRD	1.2E+04	5	2	5	-	-	-	-	-	0.0

† >, detection limit;

*39AC, control experiment 39A;

#8T, number of mice positive for cysts or trophozoites on day 8;

NSR, North Saskatchewan River water;

GVRD, Greater Vancouver Regional District water.

Table H.5 Summary of results from experiments 18 and 24: Number of C3H/HeN mice infected with *G. muris* as a function of days after infection and inoculum size (pH 6.9; 22°C; 50 mL reactor).

Trial	Residual Ozone at 5 min (mg/L)	No. of mice per trial	Inoculum (cysts per mouse)	Days After Infection (No. of mice positive for cysts)							Inactivation (log N/N_0)†
				3	4	6	8	10	12	20T#	
18-1	0.8	3	1.6E+02	-	0	2	2	2	2	2	2.3
18-2	1.7	3	3.5E+01	-	0	0	0	0	0	0	>4
18-3	4.4	3	9.0E+00	-	0	0	0	0	0	0	>4
18-C	-	1	1.3E+04	-	1	1	-	-	-	-	0
24-1	0.2	3	4.4E+04	-	0	3	3	-	-	-	3.1
24-2	0.8	3	1.3E+04	-	0	2	2	2	2	2	3.3
24-3	1.4	3	9.9E+03	-	0	2	2	2	2	2	3.3
24-C	-	1	1.4E+05	1*	1	-	-	-	-	-	0

C, control;

† >, detection limit;

#20T, number of mice positive for cysts or trophozoites on day 20.

* mice were not checked on day 3, however based on the cyst load on day 4 as well as the number of cysts in the inoculum they would have been positive on day 3.

APPENDIX I: CALCULATION OF OZONE UTILIZED PER *G. MURIS* CYST DURING OZONATION REACTIONS IN SELECTED EXPERIMENTS

Table I.1 Calculation of ozone utilized per *G. muris* cyst during ozonation reactions in selected experiments.

Trial	Applied Ozone (mg/L)	Residual Ozone (mg/L)	Utilized Ozone (mg/L)	Liquid Volume in Reactor (mL)	No. of Cysts in Reactor	Ozone Utilized per Cyst (ng/cyst)*	Inactivation (log N/N_O)†
18-1	1.39	0.79	0.60	40	3.4E+05	0.071	2.3
18-2	2.71	1.72	0.99	40	3.4E+05	0.116	>4.0
18-3	5.82	4.37	1.45	40	3.4E+05	0.176	>4.0
24-1	0.70	0.19	0.51	40	2.0E+06	0.010	3.1
24-2	1.41	0.82	0.59	40	2.0E+06	0.012	3.3
24-3	2.35	1.43	0.92	40	2.0E+06	0.018	3.3
33-A1	0.62	0.30	0.32	209.5	2.0E+06	0.034	4.2
33-A2	0.64	0.40	0.24	217.5	2.0E+06	0.026	4.2
33-B1	0.26	0.10	0.16	204.4	2.3E+06	0.014	3.1
33-B2	0.30	0.10	0.20	209.4	2.3E+06	0.018	3.1
33-C1	2.55	1.30	1.25	235.0	2.2E+06	0.134	4.4
33-C2	0.13	0.04	0.09	202.5	2.2E+06	0.008	1.2
33-D1	0.28	0.20	0.08	199.0	2.0E+06	0.008	2.2
39A-1	0.96	0.25	0.71	186.0	2.0E+06	0.066	2.7
39A-2	2.58	0.70	1.88	193.0	2.0E+06	0.181	3.7
39A-3	2.70	0.83	1.87	199.0	2.0E+06	0.186	>5.0
39A-4	3.03	0.24	2.79	196.0	2.0E+06	0.273	2.0
39C-1	2.10	0.65	1.45	190.9	2.0E+06	0.138	4.1
39C-2	1.09	0.15	0.94	185.8	2.0E+06	0.087	2.1
39C-3	2.62	0.35	2.27	193.6	2.0E+06	0.220	>5.1
39C-4	2.37	0.83	1.54	192.0	2.0E+06	0.148	3.1
39D-1	1.28	0.37	0.91	207.8	2.0E+06	0.095	3.1
39D-2	1.66	0.76	0.90	209.4	2.0E+06	0.094	3.1
39D-3	2.05	0.77	1.28	212.0	2.0E+06	0.136	3.0
39D-4	1.71	0.34	1.37	209.8	2.0E+06	0.144	3.3

C, control;
*ng/cyst, nanogram of ozone per *G. muris* cyst;
† >, detection limit of C3H/HeN mouse model.

APPENDIX J: SUMMARY OF *G. MURIS* CYST INACTIVATION DATA FOR MODELLING

Table J.1 Summary of *G. muris* cyst inactivation data for modelling.

Trial	Residual Ozone C_r (mg/L)	Utilized Ozone C_u (mg/L)	Ozone Demand C_d (mg/L)	Utilized less Demand C_u-C_d (mg/L)	Average Ozone C_{av}# (mg/L)	DOC (mg/L)	$\frac{C_u-C_d}{\sqrt{DOC}}$	Inactivation (log N/N_0)
33-A1	0.30	0.32	-	0.32	0.44	0.09	1.07	4.2
33-A2	0.40	0.24	-	0.24	0.50	0.09	0.80	4.2
33-B1	0.10	0.16	-	0.16	0.16	0.09	0.53	3.1
33-B2	0.10	0.20	-	0.20	0.18	0.09	0.67	3.1
33-C1	1.30	1.25	-	1.25	1.90	0.09	4.17	4.4
36-1	0.35	0.24	-	0.24	0.45	0.09	0.80	2.1
36-2	0.35	0.24	-	0.24	0.45	0.09	0.80	>5.1
36-3	0.40	0.33	-	0.33	0.54	0.09	1.10	4.5
36-4	0.36	0.46	-	0.46	0.57	0.09	1.53	>5.1
36-5	0.37	0.29	-	0.29	0.49	0.09	0.97	3.6
36-6	0.37	0.34	-	0.34	0.52	0.09	1.13	3.6
41-3	0.38	0.09	-	0.09	0.40	0.09	0.30	3.8
41-4	0.41	0.07	-	0.07	0.42	0.09	0.23	3.1
41-5	0.42	0.35	-	0.35	0.57	0.09	1.17	3.5
41-6	0.41	0.12	-	0.12	0.45	0.09	0.40	3.2
39A-1	0.25	0.71	0.32	0.39	0.45	0.99	0.39	2.7
39A-2	0.70	1.88	0.89	0.99	1.20	0.99	0.99	3.7
39A-3	0.83	1.87	0.55	1.32	1.49	0.99	1.33	>5.0
39A-4	0.24	2.79	1.54	1.25	0.87	0.99	1.26	2.0
39C-1	0.65	1.45	0.80	0.65	0.98	9.15	0.21	4.1
39C-3	0.35	2.27	1.37	0.90	0.80	9.15	0.30	>5.1
39D-1	0.37	0.91	0.37	0.54	0.64	9.15	0.18	3.1
39D-2	0.76	0.90	0.30	0.60	1.06	9.15	0.20	3.1
39D-3	0.77	1.28	0.49	0.79	1.17	9.15	0.26	3.0
39D-4	0.34	1.37	0.50	0.87	0.78	9.15	0.29	3.3
40-1	0.21	1.26	1.20*	0.06	0.24	1.62	0.05	2.7
40-2	0.35	2.09	1.20*	0.89	0.80	1.62	0.70	3.6
40-3	0.80	2.87	1.20*	1.67	1.64	1.62	1.31	>5.0
40-4	1.23	3.72	1.20*	2.52	2.49	1.62	1.98	>5.0

# C_{av},	$C_{av} = C_r + 0.5 \times (C_u - C_d)$
*,	estimate;
>,	detection limit of C3H/HeN mouse model.

APPENDIX K: MODEL DIAGNOSTIC ANALYSIS

K.1 $$\log \frac{N}{N_o} = -[\theta_1 C_r^{\theta_2} + \theta_3(C_u - C_d)^{\theta_4} + \{\theta_1 C_r^{\theta_2}\} \times \{\theta_3(C_u - C_d)^{\theta_4}\}]$$

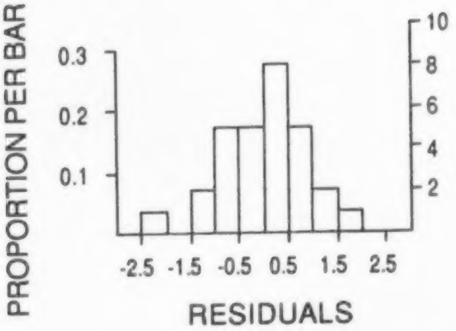
Least absolute value loss function is 17.540. Quasi-Newton estimation.

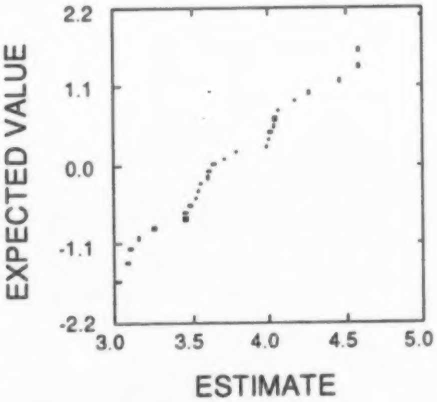
Parameter	Estimate	A.S.E.	Lower <95%>	Upper
θ_1	1.960	0.130	1.692	2.228
θ_2	0.089	0.056	-0.026	0.203
θ_3	0.816	0.038	0.739	0.894
θ_4	0.217	0.031	0.152	0.282

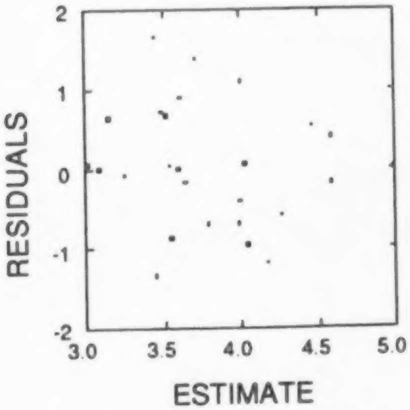
A.S.E. = asymptotic square error

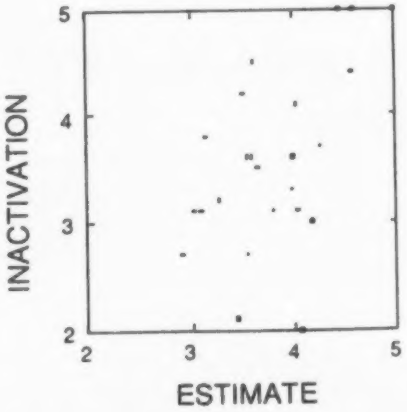
Asymptotic correlation matrix of parameters:

	θ_1	θ_2	θ_3	θ_4
θ_1	1.000			
θ_2	0.860	1.000		
θ_3	0.008	0.292	1.000	
θ_4	0.260	-0.024	0.276	1.000









K.2 $$\log \frac{N}{N_o} = -\left[\theta_1 C_r^{\theta_2} + \theta_3\left(\frac{C_u - C_d}{\sqrt{DOC}}\right)^{\theta_4} + \{\theta_1 C_r^{\theta_2}\} \times \{\theta_3\left(\frac{C_u - C_d}{\sqrt{DOC}}\right)^{\theta_4}\}\right]$$

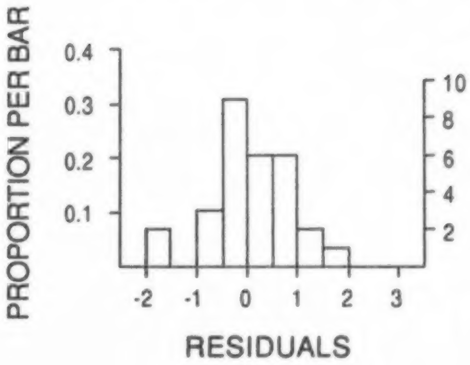
Least absolute value loss function is 16.252. Quasi-Newton estimation.

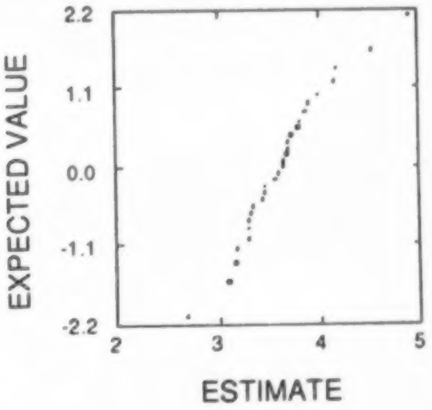
Parameter	Estimate	A.S.E.	Lower	<95%>	Upper
θ_1	1.442	0.101	1.233		1.651
θ_2	0.142	0.021	0.098		0.186
θ_3	1.109	0.071	0.962		1.256
θ_4	0.148	0.028	0.090		0.205

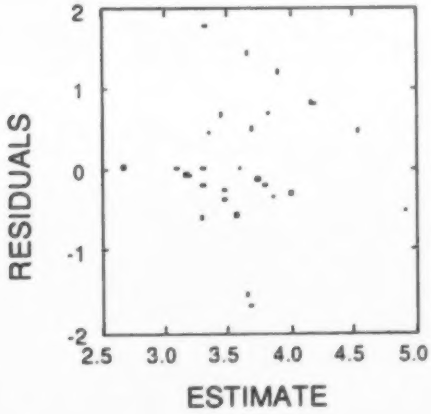
A.S.E. = asymptotic square error

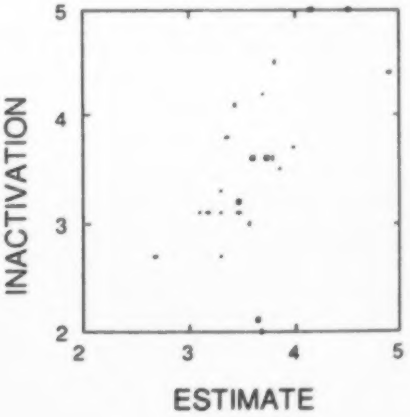
Asymptotic correlation matrix of parameters:

	θ_1	θ_2	θ_3	θ_4
θ_1	1.000			
θ_2	0.389	1.000		
θ_3	-0.896	-0.097	1.000	
θ_4	0.285	-0.014	-0.035	1.000









APPENDIX L: VITA

VITA

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PUBLICATIONS

Adamowski, K. and Labatiuk, C. 1986. Estimation of flood frequencies by nonparametric density procedures. (In) Proceedings, International Symposium on Flood Frequency and Risk Analyses, Louisiana State University, Baton Rouge, Louisiana, ed. V. P. Singh, D. Reidel Publishing Co., Dordrecht, Holland, 97-106.

Labatiuk, C. and Adamowski, K. 1987. Application of nonparametric density estimation to computation of flood magnitude/frequency. (In) Advances in Statistical Sciences, Volume 4 Stochastic Hydrology, eds. I. B. MacNeill and G. J. Umphrey, D. Reidel Publishing Co., Dordrecht, Holland, 161-180.

Labatiuk, C.W., Finch, G.R. and Belosevic, M. 1991. Comparison of a fluorogenic dye and animal infectivity for viability determination of *Giardia* following ozone disinfection. (In) Proceedings, 1990 Water Quality Technology Conference, San Diego, CA, part 2. American Water Works Association, Denver, CO, 789-803.

Labatiuk, C.W. and Finch, G.R. 1991. Ozone inactivation of *Giardia* using quantal response in animal models as a measure of disinfection performance. (In) Proceedings, Tenth Ozone World Congress, volume 2. International Ozone Association, Instaprint S.A., Tours, France, 49-58.

Labatiuk, C.W., Schaefer III, F.W., Finch, G.R. and Belosevic, M. 1991. Comparison of animal infectivity, excystation and fluorogenic dye as measures of *Giardia muris* cyst inactivation by ozone. Applied and Environmental Microbiology **57**:3187-3192.

Labatiuk, C.W., Belosevic, M. and Finch, G.R. 1992. Evaluation of high level ozone inactivation of *Giardia muris* using an animal infectivity model. Ozone: Science & Engineering **14**:1-12.

Labatiuk, C.W., Belosevic, M. and Finch, G.R. 1992. Factors influencing the infectivity of *Giardia muris* cysts following ozone inactivation in laboratory and natural waters. Water Research **26**:733-743.

Finch, G.R., Labatiuk, C.W., Helmer, R.D. and Belosevic, M. 1992. Ozone and ozone–peroxide disinfection of *Giardia* and viruses. American Water Works Association Research Foundation, Denver, CO.